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PROCEEDINGS

SPRI 2006

CONFERENCE ON SUGAR PROCESSING RESEARCH

Frontiers in Sugar Processing

SEPTEMBER 17-20, 2006
ÁGUAS DE SÃO PEDRO, BRAZIL

PROCEEDINGS OF THE 2006 SUGAR PROCESSING RESEARCH CONFERENCE

Frontiers in Sugar Processing

September 17 - 20, 2006
Águas de São Pedro, Brazil

Sponsored by
Sugar Processing Research Institute, Inc.
New Orleans, Louisiana

December 2006

PREFACE

The 2006 Sugar Processing Research Conference is one in a series of Conferences held in alternate years to provide a forum for the exchange of information among technical leaders of the sugar industry and to report and new and noteworthy developments. These Conferences are sponsored by Sugar Processing Research Institute, Inc. (SPRI).

The program for this Conference was arranged by Mary An Godshall. The Conference Coordinator was Xavier Miranda. These Proceedings were edited by Mary An Godshall.

Sugar Processing Research Institute, Inc., acknowledges the contribution in kind to the support of this conference by the Southern Regional Research Center, Argiculture Research Service, United States Department of Agriculture. We also gratefully acknowledge the support of Cargill, Fermentec, and São Joao Mill. Cargill provided support for a wonderful Brazilian Cultural Night, much enjoyed by all the delegates. Fementec provided support for the Opening Reception, and gave the delegates a very informative tour of their new research center. In addition, Henrique Amorim, of Fermentec, Mrs.Vera Amorim, and the staff of Fermentec provided invaluable assistance and guidance during the planning and execution of the Conference. São João Mill gave the delegates a comprehensive tour of the mill and ethanol distillery and then provided a delicious lunch for all.

The series, Proceedings of the Sugar Processing Research Conference, of which this is the thirteenth issue, continues the Proceedings of the Technical Session on Cane Sugar Refining Research, which was published every other year from 1964 to 1980. For individual copies of this volume, as well as back issues of the former series, as long as the supply lasts, write to Sugar Processing Research Institute, Inc., 1100 Robert E. Lee Boulevard, New Orleans, Louisiana 70124. Before 1986, Proceedings were published by the Agriculture Research Service, U.S. Department of Agriculture. Since 1988, Proceedings have been published by Sugar Processing Research Institute, Inc.

Mary An Godshall
Managing Director
Sugar Processing Research Institute, Inc.

José Alvarez, Chairman
Sugar Processing Research Institute, Inc.

SUGAR PROCESSING RESEARCH INSTITUTE, INC.

Sugar Processing Research Institute, Inc., is an independent, non-profit research institute supported by the international sugarcane and sugarbeet production and refining industries and their supplier and user companies. The Institute is housed at the Southern Regional Research Center of the United States Department of Agriculture, Agriculture Research Service, under a Memorandum of Understanding with USDA. The association with USDA offers many synergies for the benefit of the sugar industry. The SPRI organization is unique in that it conducts both beet and cane sugar processing research.

The history of the institute began in 1939 with formation of the Bone Char Research Project at the National Bureau of Standards in Washington, D.C., under the direction of Dr. Victor Dietz, with a mandate to study the, then new, sugar decolorizing adsorbent, bone char. In 1963, it moved to New Orleans, Louisiana, and became the Cane Sugar Refining Research Project, with Dr. Frank Carpenter as its director. At that time, the focus was exclusively on cane sugar processing issues. In 1981, under the direction of Dr. Margaret Clarke, its scope was greatly expanded when it became Sugar Processing Research, Inc, and included beet sugar and raw cane sugar manufacturing in its research portfolio. In 1991, it was renamed Sugar Processing Research Institute, Inc. Mary An Godshall became the Managing Director of the Institute in 2000. This Conference commemorated 67 years of continuous research for and service to the sugar industry by SPRI.

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This volume is

dedicated to the memory of

Dr. Frank G. Carpenter

1920 - 2006

FOREWORD

The theme of the 2006 Conference was **"Frontiers in Sugar Processing."** A wide range of topics is covered in the 46 papers within these pages, reflecting the range of developments in the sugar and allied industries over the last few years.

Mike Kearney, the winner of the 2006 SPRI Science and Technology Award, takes us into the little-explored area of process symmetries, showing how the engineering of equipment to take advantage of symmetries can result in smaller systems, better energy use and efficiency, giving examples that make these complex ideas understandable.

Included are topics relating to product innovation, new analytical techniques, process innovations, and energy production.

The first section of the Proceedings deals with over-all technological and societal issues, including ethanol production in Brazil, the status of biotechnology in the sugar industry, the sugar situation in Europe, emerging food trends that affect the sugar industry, an overview of the new sweeteners that compete with sucrose, and organic sugar. Papers on the direct production of white sugar in a cane sugar mill and on the stringent demands for quality improvement in raw sugar show the converging needs for ultimate quality at all levels of sugar production.

Color control continues as an important focus, and it is covered by several papers, including one that looks at the effect of enzymes on reducing color and one on modeling color behavior. Process improvements leading to color reduction are discussed, as well as other process innovations leading to reduction in the use of processing aids and improved circulating efficiency in vacuum pans.

Research innovations include a paper that discusses the use of mannitol as a sensitive indicator of sugarcane deterioration and bacterial contamination in fuel alcohol production, with development of a concurrent simple test for mannitol. A good indicator of sugarcane deterioration has been needed for a long time, and this research seems to have met that need. Another paper discusses the discovery of filter clogging material in raw and white cane sugar.

For the first time, the SPRI conferences had a section featuring commercial products – short papers in which a new product or process could be introduced to the audience. In this session we learned about the Dedini Direct Refined process for production of refined sugar, optimization of natural biocides derived from hops, specially designed screens to remove bagacillo for improvement in juice quality, natural products for cane juice disinfection and clarification, and commercial pesticide residue analysis, a hitherto very difficult analysis, little applied to sugar.

These proceedings also contain an invited paper by Dr. Grant DuBois on beverage standards and testing and trends in sugar quality for beverages. This previously unpublished paper was presented at a SPRI Workshop and fits very well into the topics at hand.

Many other subjects are covered in these proceedings, and we hope that the reader will find much of interest in these pages.

Mary An Godshall
October 2006

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In Memoriam

Dr. Frank G. Carpenter

(1920-2006)

Dr. Frank G. Carpenter, a famous sugar technologist, and mentor to many, passed away Thursday, November 16, 2006, in Lafayette, Louisiana, at the age of 86.

Dr. Carpenter was born in 1920 in Washington, D.C. He was a long time resident of New Orleans, Louisiana, and moved to Lafayette after Hurricane Katrina destroyed his home of 43 years.

Dr. Carpenter served in the U.S. Army during World War II, where he received injuries to an ankle in a plane crash. After the war he earned a PH.D. in Chemical Engineering at the University of Delaware and began his career at the U.S. Bureau of Standards where he joined the Bone Char Research Project in 1948, under the direction of Dr. Victor Dietz, with a mandate to study bone char, at that time a new sugar decolorizing adsorbent Dr. Carpenter developed a huge fund of information about sugar color and the mechanism of bone char decolorization, setting the standard for all future work on sugar color. This work is gathered in the Technical Reports and Proceedings of the Bone Char Research Project.

In 1963, when the bone char project was completed, he moved to New Orleans to initiate and direct the Cane Sugar Refining Research Project (CSRRP), in cooperation with the U.S. Department of Agriculture and an international consortium of sugar refiners. Dr. Carpenter continued an illustrious career in sugar technology until his retirement in 1981. During his time with CSRRP, work was initiated on identifying the minor components in sugar and their effect on color and processing, understanding the nature of acid beverage floc, and on the flavor of sugar products. At his retirement, the Cane Sugar Refining Research Project became Sugar Processing Research Institute.

Dr. Carpenter was very active in ICUMSA, SIT, and the US National Committee on Sugar Analysis. He was an early proponent of systematic methods development and method acceptance within ICUMSA. He was a mentor to all he met and loved to "tinker" with the analytical equipment to fine-tune it and to make it better. He was of the old school, before scientific equipment did most of the work, and in the early days, he built his own gas chromatograph and packed his own columns.

Dr. Carpenter loved New Orleans and the wonderful friends and neighbors he had there. He was a very devoted family man, he was deeply religious, he loved to go camping, and he loved to tell wild stories. He was a man of absolute integrity, faithfulness and with a marvelous sense of humor.

Dr. Carpenter is survived by his wife of 61 years, Angela, three daughters and two sons.

2006 SPRI Science and Technology Award

MIKE KEARNEY

Mike Kearney, Director of New Technology at Amalgamated Research Inc., was born in Utah in 1952. He received a Bachelor's Degree in chemistry from Weber State University and subsequently studied physical chemistry at the University of Nevada. He has 28 years of experience in process R&D. His general background is in the development of innovative technology and directing such technology from lab and pilot-scale to full-scale implementation.
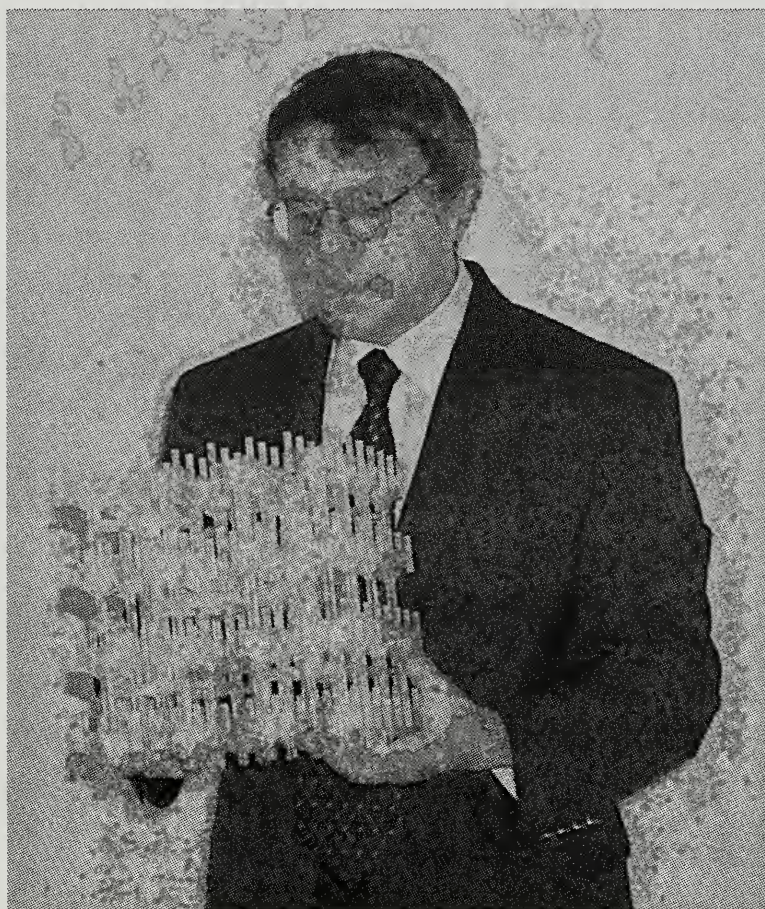
His work has included contributions to the development of the first simulated moving bed molasses separator used in the beet industry. Extending this technology, he and his colleagues developed the raw juice chromatography process as an alternative to conventional beet and cane processing.

Personally interesting projects have included the design of neural networks for agricultural forecasts and the use of evolutionary computation to evolve solutions to difficult chromatography problems.

Mike originated the general concepts for using fractal based technology as an alternative to fluid turbulence and for the efficient control of fluid processes. Processes or phenomena which demonstrate poorly controlled or turbulent fluid characteristics generally benefit from this technology. Presently fractal technology is being used in the sugar industry for improving chromatography and ion exchange and for controlling sugar silo air flow. Apart from sugar processing, the technology is being evaluated and applied in the petroleum, mining, biomass and water treatment industries.

Mike is presently interested in the effects of process symmetries on energy use. He is a member of the ACS and the AIChE.

The photograph shows Mike with a fractal structure used for fluid distribution.

SPRI SCIENCE AND TECHNOLOGY AWARD

The SPRI Science and Technology Award is presented biennially to an outstanding scientist, whose research accomplishments are distinguished by their originality and their contribution to sugar processing and production. The Award is presented for the purpose of promoting science and technology in sugar processing and production.

Previous Winners of the SPRI Science Award

2004 **Mohamed Mathlouthi**
Water-Sucrose Interactions, Quality of Crystals and the Storage Stability of Sucrose

2002 **Benjamin L. Legendre**
The Quest for Quality in Louisiana Sugarcane and Sugar

2000 **Jean-François Thibault**
New Ways to Add Value to Sugar Beet Pulp

1998 **Markwart Kunz**
Sucrose - Raw Material for Chemistry and Biochemistry

1996 **Pascal A. Christodoulou**
Energy Economy Optimization in the Separation Processes of Sucrose-Water and Non-Sugars

1994 **Frieder Lichtenthaler**
Computer Simulation of Chemical and Biological Properties of Sucrose, the Cyclodextrins and Amylose

1992 **Riaz Khan**
Chemical and Enzymic Transformations of Sucrose

1990 **Giorgio Mantovani**
Growth and Morphology of the Sucrose Crystal

1988 **Leslie Hough**
Sucrose, Sweetness and Sucralose

1986 **Andrew Van Hook**
Events in Sugar Crystallization

Chairman's Welcome and Introductory Remarks

It is my pleasure to welcome you to the 2006 SPRI Conference on Sugar Processing Research. The attendees here are from 23 countries, representing 59 companies. You are a diverse group, as befits the theme of this conference, Frontiers in Sugar Processing.

What is happening today in research and development in the sweetener industry? Where is it headed? Where should it go? In this new century, the Millennium, we continue to see a decline in support for research. Processing research centers are losing funding from traditional sources – mills and factories – and are looking to external funding. At the same time, the research focus is switching from sugar processing research to ethanol, biotechnology and new products.

These factors represent both challenges and opportunities. With the notable exception of Sugar Processing Research Institute (SPRI), research institutes tend to be country-specific, but we may see that begin to change. We will also see research branch into related fields. In the meantime, however, the lack of funding limits the scope of research that can be done.

Multiple approaches are needed for the survival of quality research in the sugar industry. Funding will have to come from a wider network of sources, including subscribed members, government, industrial grants and contracts. Research centers will also become centers for training, extension, troubleshooting and information and technology transfer. At the same time, they will need to balance these functions with doing new research, which, again, must be balanced between pre-competitive research (that which can be shared with members or with the wider community via publication) and proprietary research, which is funded by a specific entity (or entities) for a specific purpose. Strategic alliances among different groups can enhance the strength of all involved. In a strategic alliance, each partner brings something important and unique to the table. Above all, fresh and innovative ideas are needed.

What is the future of research in the sweetener industry? In 1998, M. Yalpani said that carbohydrate technology is the sleeping giant of the next century; we are now in that new century. We know that most types of chemicals produced from petroleum can be produced from carbohydrates, and the grain/corn industry is making great strides in that direction. In a recent survey of sugar industry managers, all respondents said their company was interested in sugar cane or sugar beet bio-products and/or biomass utilization. The products of greatest interest were plastics from sugar, sucrochemistry, ethanol production, vinasse applications, beet biomass utilization, and biomedical applications. In this day and age, biotechnology and new products from sugar processing are a necessary direction for research and continued profitability.

As you enjoy the papers and posters over the next few days, please keep in mind the importance of quality research to the continued strength and viability of our industry.

Robert O. Hatch
Chairman of the Board of SPRI

Engineered Symmetries Force Process Efficiencies

Mike Kearney
Amalgamated Research Inc.,
Twin Falls, Idaho, USA

Introduction

The goal of this paper is to present a new general approach to understanding and implementing process efficiencies. This approach is based on the concept of symmetry. Results can be used to improve processes and to drive innovations.

Symmetry Discussion

Is there a general analytical tool which is applicable to many of the diverse aspects of process efficiency? This paper will argue that symmetry provides such a tool. Symmetry can be defined as immunity to possible change. Figure 1 illustrates this concept with examples of plane symmetries.
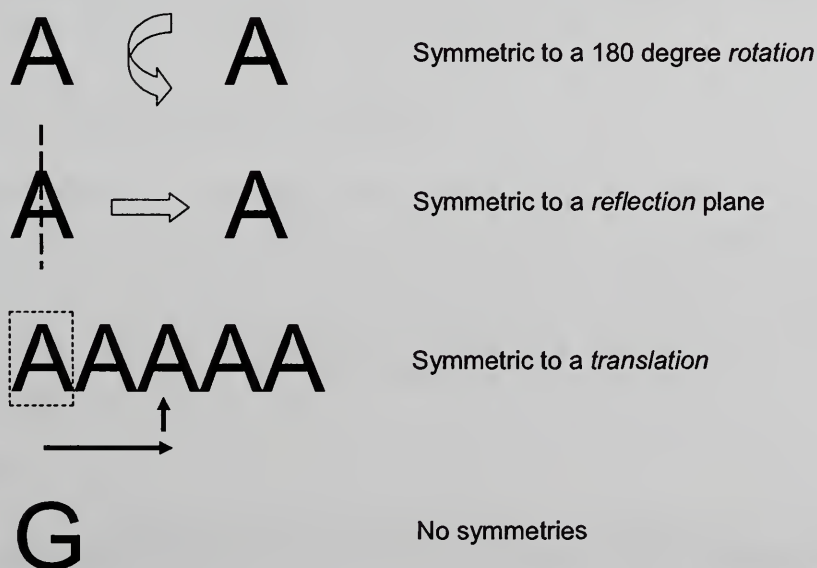
Figure 1. Example of plane symmetries.

Another example is illustrated in Figure 2. In this case, a checkerboard pattern increases in symmetry as smaller and smaller squares are used for the pattern.
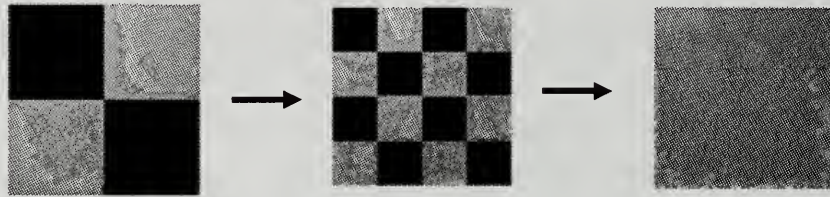
Figure 2. Checkerboard symmetry increases as squares are made
smaller. The number of possible symmetry operations which leave
the checkerboard the same increases. At infinitely small squares,
the checkerboard is completely symmetric to all translations for any
arbitrarily small square size.

Symmetry applies not only to geometric objects, but rather, to any characteristic under consideration. For example, a sine wave exhibits symmetry with to respect time. A symmetric translation of the wave from trough to trough can be made (Figure 3).
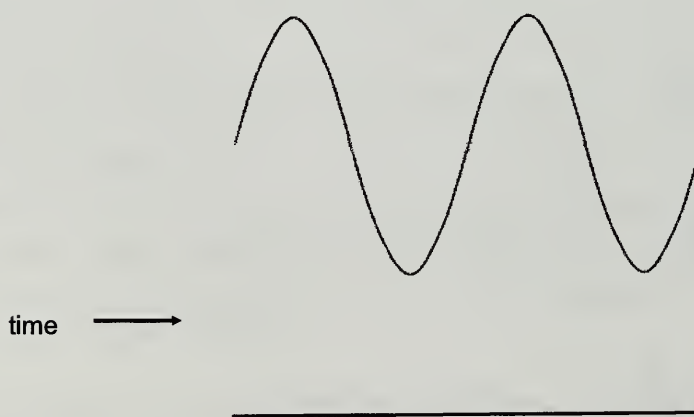
time ⟶

Figure 3. A sine wave exhibits symmetry over time, but less symmetry
than the straight line which is symmetric to translation of any point to any
other point.

As another disparate example, a very useful symmetry to an astrophysicist is symmetry of the Universe with respect to the laws of nature. A laboratory transported to any location in the Universe would determine the same laws. In general, a particular aspect of a situation can be chosen and if this particular aspect remains the same under a change, the situation is symmetric under the change with respect to that aspect (1).

Process Efficiencies

For this paper, process refers to any process or series of actions in general, not only engineering unit processes. Efficiency improvements are considered to cover a broad range and include, among others:

Less energy use
Smaller equipment
Ease of operation
Higher productivity
Lower costs
Reduced material handling
Ease of process scale-up
Simpler process control hardware and software
Simpler process analysis and modeling
Simpler process description and understanding
Reduction of human/computer time and effort

Process Elements

For this paper, process elements are defined as any disparate aspects of a process which can be evaluated with symmetry concepts. This is essentially anything to do with the process. For example, processing elements may be flow configuration, control software, equipment layout, chemical media, operating mode, etc.

Symmetry Analysis of a Process

What does the alteration of aspects of a situation such that the result is the same as the initial situation have to do with process efficiency? To understand this, an argument will be made with the use of a specific example. Symmetries will be described for elements of an ion exchange system. For each symmetry, the specific, ordinarily understood explanation for the process improvement is described. In each case the improvement is a symmetry increase.

1. Vessel flow

Figure 4 illustrates two possible ion exchange column configurations. The small dark rectangles represent some type of fluid distributor and collector. The figure to the left represents the ion exchange column operating with flow in the horizontal direction while

in the column to the right the flow is vertical. In the column to the left, without high pressure drop, the flow will be uneven. The ion exchange resin could also classify in an undesirable way. The purpose of this example is to demonstrate that even in this obvious case the much preferred configuration of the flow is one in which the process exhibits symmetry. The preferred vessel flow configuration is one which is symmetric with respect to the gravitational field.
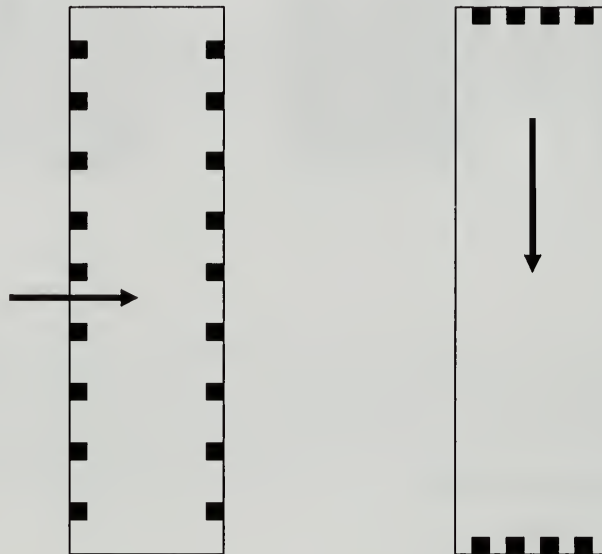
Figure 4. Possible flow directions in a vessel.

Symmetry increase 1: Vessel flow symmetry

Process efficiency in vessel design is increased by configuring the vessel with flow symmetric with respect to the gravitational field.

2. Distributor flow

Conventional fluid distributors and collectors, such as radial or lateral types, are generally designed using pressure drop considerations. Figure 5, to the left, illustrates such a distributor. In this case flow travels to orifices located along the laterals. These devices have different path length and hydraulics to the various orifices. They operate best over a narrow range of flow rates. Because ion exchange involves steps at variable flow rates, this can be detrimental to operation. Also, at too slow flow rate, the majority of the liquid may exit the orifices closest to the main plenum. Further, exiting fluid will exhibit a time lag between exit points due to the variable path length to exit points so the distributed fluids can be somewhat "bowed" as they enter the system. This can result in a spreading of concentrations of the exiting fluids. For example, the waste regenerant may be more dilute and a larger volume than necessary.

Such devices can be replaced by a distributor which depends upon symmetry rather than the expenditure of energy. Figure 5, to the right, illustrates a simple symmetric distributor. Fluid enters the center of the device and travels with equal path length and hydraulic symmetry to the eight exit points. The device is not dependent upon flow rate and pressure drop can be negligible. (Note: Only the hydraulic symmetry concept is illustrated, fewer exits are shown for the symmetric distributor - this can be increased using more symmetric "legs").
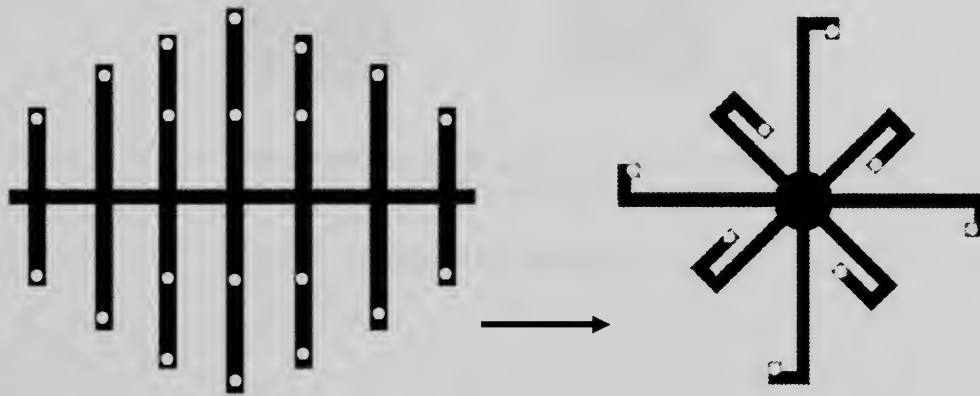
Figure 5. An asymmetric distributor is replaced with a hydraulically symmetric distributor in order to provide flow equally to all exits and to eliminate energy use.

Symmetry Increase 2: Distributor hydraulic symmetry

Process efficiency due to distributor performance is increased by using a distributor based on symmetry rather than pressure drop. A very large flow turndown is provided, distributor "bowing" caused by time lag to exit points is eliminated and energy use is reduced.

3. Resin size

Monospheric resins are preferred for highest efficiency. A reason is that the diffusion path length in a population of monospheric resin will be the same so that the exiting profiles, such as regenerant rinse, will be relatively sharp compared with those obtained using a resin population containing a variety of bead size. The result can be less dilute liquid fractions and less liquid quantities. This can save evaporation costs, handling/ transportation costs, downstream equipment size etc. A monospheric population of beads is symmetric with respect to bead size. Figure 6 illustrates bead populations with a large spread of bead size and to the right, a monosphere population.
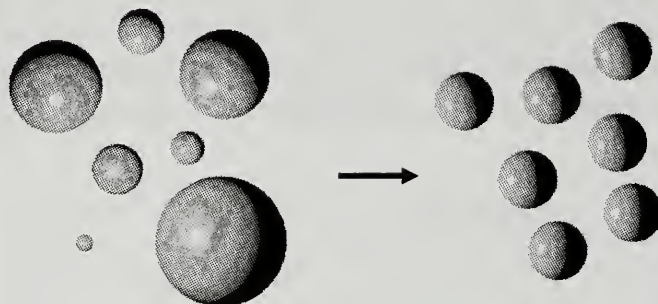
Figure 6. The resin population to the left is asymmetric with respect to size. The population to the right is symmetric to size.

Symmetry Increase 3: Resin size symmetry

Process efficiency related to product quantity and concentration is increased by using a resin symmetric to size. Pressure drop is also reduced.

4. Resin-void

An ion exchange resin can be made more efficient by using smaller particle size. Because of shorter diffusion path and higher surface area, kinetics are quicker and a smaller amount of resin can be used with shorter cycle time. System size can be decreased. The smaller resin size can also provide sharper exiting stream profiles. This leads to more concentrated material and less quantity. These are energy and handling reductions.

A smaller bead population has greater resin-void symmetry than a larger bead population. This can be understood by comparing the large and small bead populations in Figure 7. The small bead population has many more possible bead translations which will leave the population the same. This increase in symmetry can also be recognized by comparison with increasing checkerboard symmetry.
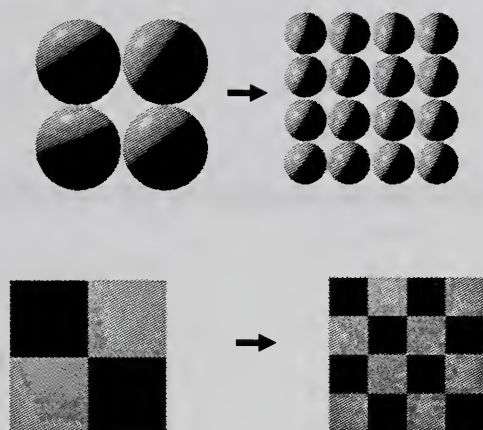
Figure 7. Resin-void symmetry increase.

Symmetry Increase 4: Resin-void symmetry

Process efficiency related to product quality and quantity and energy use is improved by increasing the resin-void symmetry.

5. Resin bed pressure

The system improvements discussed to this point tend to drive the design towards implementation of another symmetry. Although the system has very fast kinetics, primarily due to the small resin, it may not be possible to take advantage of the configuration due to high pressure drop developed as the specific processing rate is increased. By using smaller resin and higher flow rate, a pressure asymmetry will be forced on the resin bed (pressure drop). It will be necessary to consider adding another symmetry in order to continue the optimization.

How is a symmetry added to the now problematic pressure profile of the resin bed? One way pressure symmetry can be imposed is by spreading out the resin in a flat , horizontal configuration. For the same specific flow rate, this reduces the linear velocity and provides a shorter distance for pressure drop to develop. The bed will now exhibit close to the same pressure at any resin bed location. Therefore, pressure drop does not exist. Figure 8 illustrates the reduction of bed depth and subsequent increase in resin bed pressure symmetry. A sample of pressure taken anywhere in the short resin bed can be expected to be about the same as the pressure taken anywhere else in the bed.

P = 50 psi

P = 0.5 psi

delta P = 50 psi

P = 0.4 psi

delta P = 0.1 psi

P = 0 psi
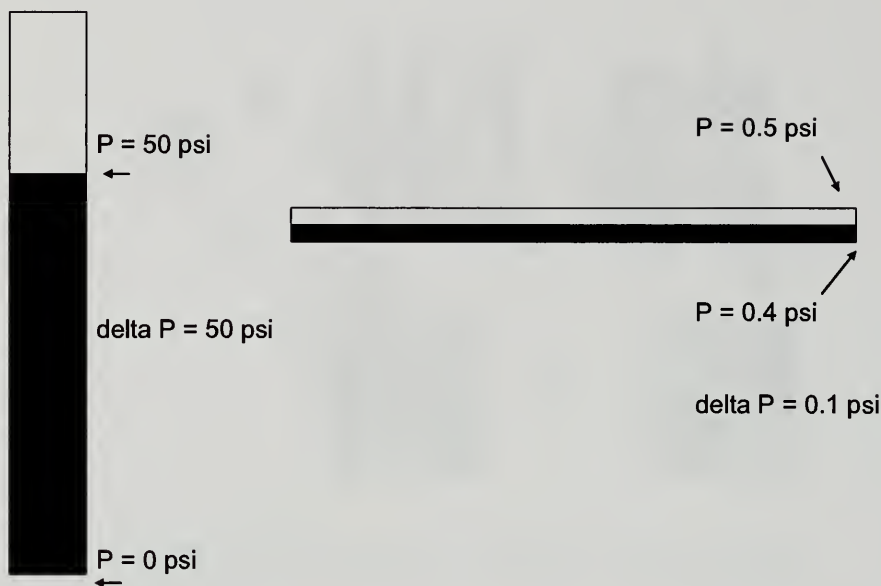
Figure 8. Pressure symmetry is increased in an ion exchange resin bed by using a spread-out flat configuration.

Symmetry Increase 5: Bed pressure symmetry

Process efficiency related to bed pressure drop is increased due to an increase in bed pressure symmetry. The system can operate with less pressure drop so the ion exchange system can operate at a higher specific flow rate (flow rate per unit of resin), system size is reduced and less energy is used. Overall system pressure is lower so the vessel can use thinner walls and less structural support - reducing material and construction costs.

6. Fluid momentum and turbulence

As bed depth is reduced to take advantage of smaller resin, the effect of poor distribution becomes critical. Flow may channel or resin dead spots may exist due to a poor coverage of the bed by a small number of distributor exits. Turbulence due to jetting of the high flow from distributor exits will disturb the bed and cause leakage and a spreading of exiting concentration profiles. High flow rate through the small number of exits in the symmetric distributor of Figure 5 will certainly exhibit these problems. Additional symmetry is now needed for this process element. In particular, the flow momentum within the column should be symmetric.

This problem can be addressed by adding additional smaller iterations of symmetric structure to an existing symmetric distributor. Figure 9 illustrates the progressive construction of smaller scale structure added to an initial "H" type symmetric distributor (the "H" type is easier to illustrate than the symmetric distributor in figure 5). This provides an increase in fluid momentum symmetry over the exit plane of the distributor. Symmetry operations - rotations, reflections and translations are used for this

construction. Figure 10 illustrates the increasing symmetry of the fluid flow from the exit plane of the distributor.
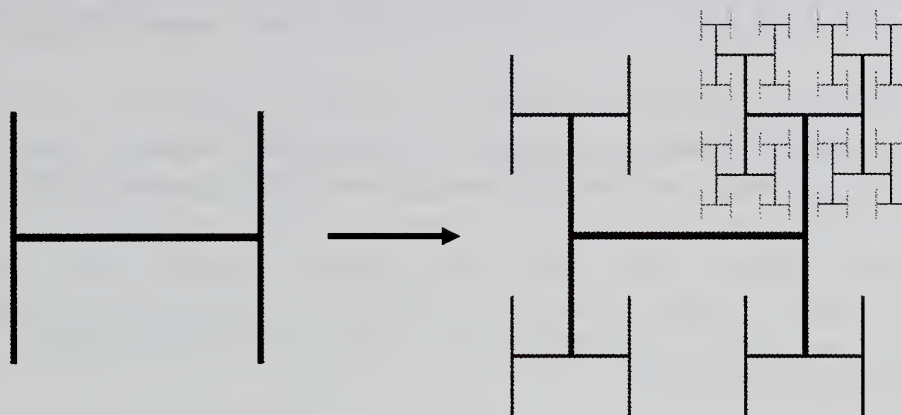
Figure 9. The progressive increase in distributor symmetry by addition of smaller scale symmetric structure. The result is a fractal.
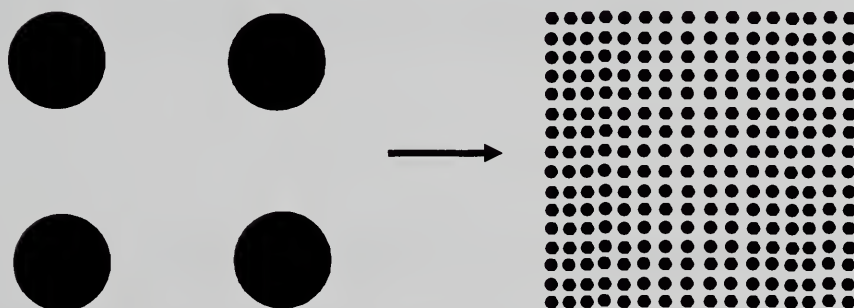
Figure 10. As smaller scale symmetric structure is added to the distributor, the exit plane flow symmetry increases (compare checkerboard symmetry). The size of dots represents fluid momentum from each exit (not the size of an exit). In this example, the exiting momentum from an exit point is reduced by the factor 1/64. As a result, turbulence is significantly reduced.

Symmetry Increase 6: Flow momentum symmetry

Process efficiency related to fluid distribution can be improved by adding smaller and smaller scale hydraulically symmetric structure. The flow momentum becomes progressively more symmetric (gentle flow at any point rather than variable velocities). Turbulence - a very asymmetric and energy dissipative phenomena - is progressively eliminated.

Observation: Note that in the last two examples the motivation for introducing increased symmetries was the appearance of new asymmetries caused by symmetry additions. The use of smaller resin revealed a bed pressure asymmetry which was addressed to provide additional efficiency and the "flat" resin bed revealed a serious flow momentum asymmetry which was also addressed. This procedure of adding new symmetries to reveal asymmetries with subsequent addition of more symmetries can drive a process toward higher and higher efficiency.

7. Operating mode

Continuous systems are preferred to batch processes. The advantages include consistent product quality and ease of operation. For ion exchange the benefits also include less regenerant, less waste and higher exiting concentrations. In Figure 11 a repeating ion exchange batch process is illustrated. The measurement illustrated may be a particular flowrate or other parameter. The process exhibits symmetry over time as seen in the repeating pattern. This time based symmetry can be increased by using a continuous process. A continuous process for the same parameter is shown as a straight line below the batch process. A translation of any point on the time function can be translated to any other point. Although not ordinarily described in this manner, this is an increase in symmetry.

A repeating batch process

A continuous process - The process has increased symmetry over time
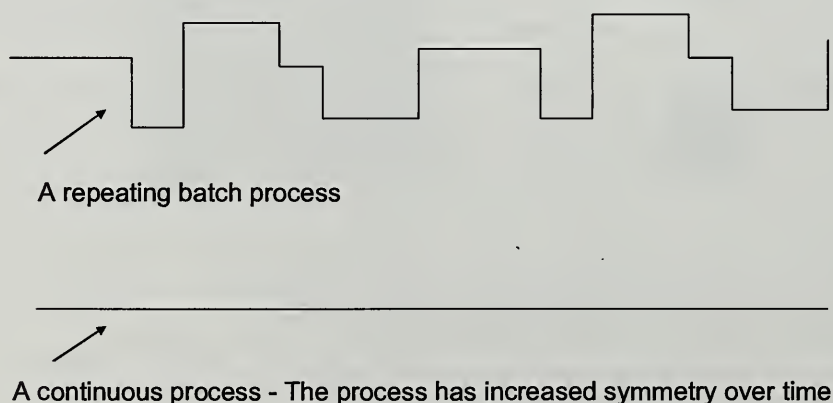
Figure 11. A comparison of batch and continuous operation symmetry.

Symmetry Increase 7: Operating mode symmetry

Process efficiency related to consistent product quality and regenerant use can be improved by increasing the symmetry of the operating mode.

8. Control loop tuning

Certainly any flow rates, tank levels etc. associated with an ion exchange system will be tuned as a matter of course. Tuning a PID loop to obtain a constant flowrate is simply the introduction of a symmetry over time. Figure 12 illustrates a tuned and untuned PID loop.

untuned PID loop

tuned PID loop
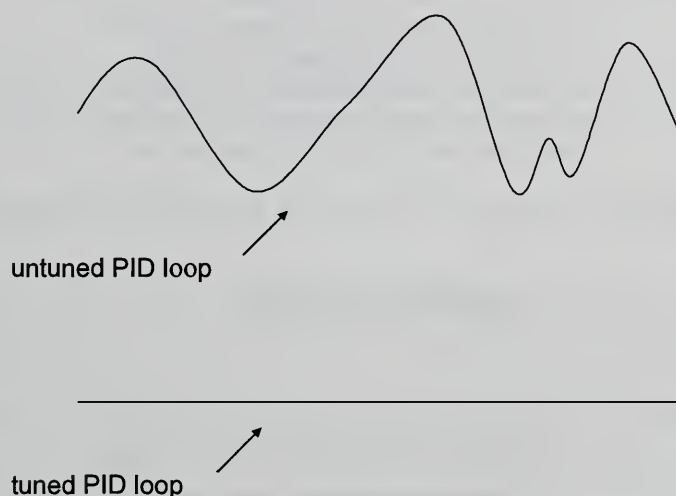
Figure 12. Tuning control loops is introducing process symmetries over time.

Symmetry Increase 8: Control loop symmetry

Process efficiency related to consistent operation can be improved by increasing the symmetry of the control loops. Additional symmetry over time is introduced.

9. System analysis and CFD modeling

Although a CFD (computational fluid dynamics) model of the original ion exchange system with large turbulent jets, large pressure gradients, channeling and other chaotic aspects would be very interesting, it would also be extremely difficult and time consuming to develop and solve. On the other hand, if the entire process were symmetric over small volumes and short periods of time, which the final improved design approaches, a relatively simple analysis can be done.

Figure 13 illustrates the relative amount of the process which must be modeled for the initial asymmetric ion exchange process versus the symmetric process.

Note that the reduction of effort is in both the spatial dimension (smaller volumes used for analysis since all characteristics of volumes are the same) and in the temporal dimension (since the process is the same at any time). This saves both human and computer effort. This is another example of how symmetries add efficiencies to the overall process - in this case, the associated analysis and modeling.

The original asymmetric process requires modeling of the entire spatial envelope. Because so many characteristics will always be changing with time, calculations must also include large time increments.

The symmetrically optimized system can be modeled with a very small slice of the process. The process is also more symmetric over time so time dependent characteristics tend to drop out of the analysis and calculations.

Figure 13. Comparison of symmetric and asymmetric analysis/modeling.

Similarly, symmetry results in the process being described in a simpler manner and it is easier to understand the process.

Symmetry Increase 9: Modeling/analysis symmetry

Process efficiency related to analysis and modeling can be improved by increasing the symmetries of the process. Analysis/calculations become simpler.
Process description is simpler and easier to understand.

10. Process scale-up

Scale-up of processes is often a difficulty. Efficiency can be lost as a process moves from lab scale to pilot scale to full scale. For example, the operation of the lateral distributor in Figure 5 becomes worse with scale-up because exit points become more and more hydraulically differentiated.

Maintaining smallest scale symmetry provides a logical path to process scale-up. The distributor in Figure 9 can be easily scaled-up by maintaining the illustrated smallest scale symmetric structure and simply adding larger scale "feeder" symmetric structure. In this case, additional "H" type structure can be added. By this means, the desired flow momentum symmetry will be maintained for any size device.

Another way to understand the use of "smallest scale symmetric structure" is with reference to Figure 13. The small slice of the symmetric process can simply be repeated to produce a device larger and larger in size while maintaining the symmetry of the original slice.

Symmetry Increase 10: Scale-up symmetry

Process efficiency related to process scale-up can be improved by maintaining smallest scale symmetries. Symmetry provides a logical path to scale-up.

A Symmetry Based Fractal Weak Cation Exchange Softener

Full scale weak cation softeners have been constructed which incorporate most of the symmetries, but not all, discussed above (2). For example, these are batch systems not continuous. These softeners typically operate with exhaustion as high as 500 bed volumes per hour. A conventional weak cation softener typically operates at about 50 bed volumes/hour. The resulting softener is about 10% the size of a conventional weak cation softener and about 2% the size of a conventional strong cation softener.

There is no measurable pressure drop across the hydraulically symmetric fractal distributors. Although specific flow rate is extremely fast, bed pressure drop is negligible. Due to fluid momentum symmetry within the vessel, there is no disturbance of the resin bed from turbulence. Internal pressures are so low that a non-coded vessel may be used and flow can be provided by a head tank rather than by pump. Processes based on symmetry principles may appear quite different in appearance compared with conventional equipment. Figure 14 illustrates a short bed "fractal softener". This unit is 5 feet across with a six inch resin bed depth. It operates at a maximum of 700 gpm.
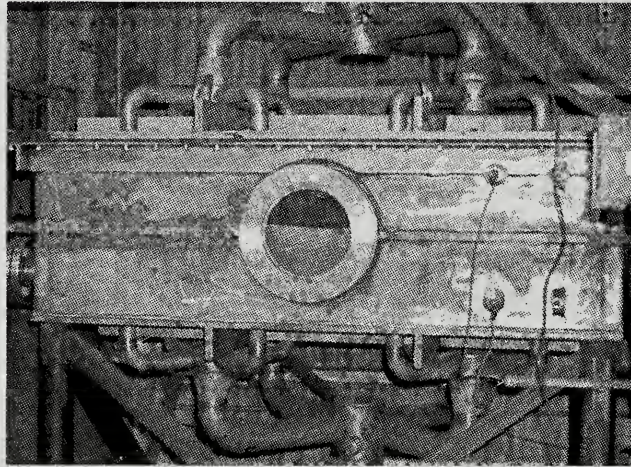
Figure 14. The fractal softener involves the use of several symmetries including bed pressure symmetry and flow momentum symmetry (Amalgamated Research Inc.).

The symmetry-efficiency connection

The ion exchange discussion demonstrates the broad connection between symmetry and process efficiency. What is generally responsible for this connection? It is helpful to consider a simple description of what the addition of symmetry does. It makes everything "the same" for the aspect of interest.

If the interest is pressure drop through a resin bed, absolute pressure symmetry of the bed, by definition, means pressure is the same everywhere so pressure drop cannot exist. Therefore energy will be saved. Absolutely symmetric PID control loops are, by definition, the same over time. Therefore the system is under control and a consistent product will be produced. An absolutely symmetric fractal distributor provides, by definition, the same exiting fluid momentum at any point on the exit plane. So there is no inhomogeniety of the flow. An absolutely symmetric monosphere resin will, by definition, exhibit the same diffusion characteristics for all beads. Therefore there will be less spreading of exiting fluid profiles and less energy will be used for concentration.

For some cases, Figure 15 provides a useful way of understanding the symmetry-efficiency connection. This figure illustrates that a less spread parameter (narrower distribution) is more symmetric to a measured value. For example, the decreasing spread could represent the exiting concentration of an ion exchange regeneration rinse profile with increasing symmetry caused by using a monosphere resin or a smaller resin size. Or it could represent measurement of profiles for calculation of theoretical plates resulting in increased efficiency in a chromatography application. Or, as in Figure 16, the profiles could represent the increasing flow symmetry and efficiency as smaller scale structure is added to a fractal distributor. Evaluating the outcome of process elements in such a manner can reveal system asymmetries and help provide an understanding of symmetry effects. The outcome is a reflection of the symmetry of the process element leading to the outcome.

Increasing symmetry

Increasing efficiency
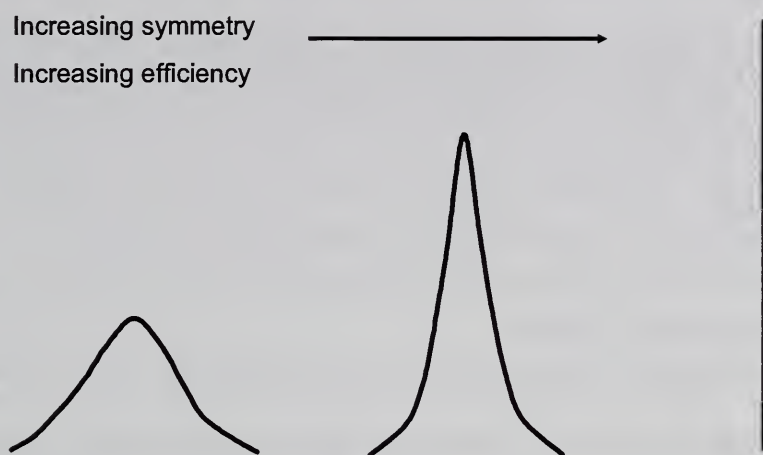
Figure 15: A less spread profile of a parameter is more symmetric to
the measured value. This can be either a spatial or temporal
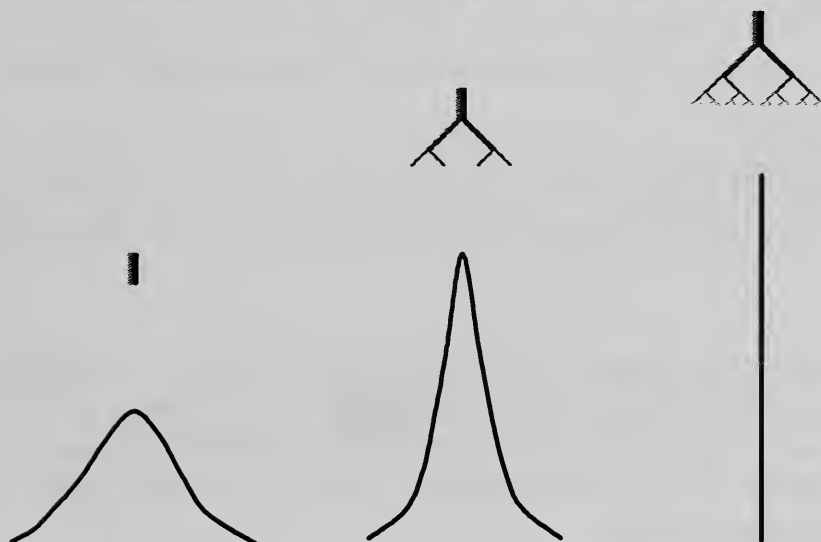
Figure 16: Increasing fractal distributor symmetry (adding
smaller scale structure) narrows the distribution of fluid
properties such as velocities (turbulence), bubble size, etc.

So far in this discussion, for every case of symmetry increase, the desired outcome of the
process element was symmetric. Later in this paper, the special case of necessary
asymmetric outcomes will be discussed.

Process Innovation - Symmetries and Process Disappearance

As an example of the innovation enabling effect of symmetries, with the fractal weak cation softener a design loop can be evaluated wherein increases in symmetry allow the resin bed to approach zero size.

First in conventional terms:

BEGIN:
Reduce resin size to obtain faster kinetics.
Reduce resin bed depth to reduce pressure drop due to the smaller resin. Do this by eliminating resin.
Add additional fractal distributor scaling for better distribution and less turbulence over the shorter bed.
Shorten cycles.
GOTO BEGIN

Stated in symmetry terms:

BEGIN:
Increase resin/void symmetry.
Increase bed pressure symmetry.
Increase fractal distributor symmetry.
Shorten cycles.
GOTO BEGIN

A practical decision must be made concerning when to stop shrinking the resin bed. Note that in the loop above, peripherals such as pipe and valves do not change in size so there is no benefit with respect to these items. Also, cycle times become shorter and shorter and there are good reasons not to exhaust the bed every half second with associated valves opening and closing so quickly.

On the other hand, and perhaps the most useful aspect of the disappearance via symmetry observation, entirely new system designs may come into play which specifically take advantage of the new symmetries and allow process intensification to continue. The use of deep symmetry allows an engineer to work within a large range of possibilities for the design of an efficient and practical device.

(Note: The "disappearance" effect is common when symmetries are added and extend to process aspects which are ordinarily not clearly connected. For example, recall the reduction in CFD/analysis effort required for the symmetric process in Figure 9).

Fluid turbulence

This section will discuss in detail a particular instance of asymmetry in order to make the argument of this paper more clear. This asymmetry is turbulence - the exemplar of

asymmetric phenomena. It is interesting, in light of the conclusions in this paper, that it is employed to obtain outcomes which are symmetric. To understand this point, refer to Figure 17. In this case, an impeller transfers large amounts of energy to a vessel for mixing components A and B. Energetic, asymmetric turbulent eddies are forced on the system. The end result is a symmetric outcome - the solution is mixed. The eddies eventually dissipate as heat. If faster mixing is desired with the same equipment, the procedure is usually to create even more energetic turbulence. With respect to efficiency, this method is in direct contradiction to the premise of this paper that a symmetric outcome can be obtained more efficiently by increasing the symmetry of the appropriate process elements.

Recall the asymmetric lateral distributor. Its design is based on pressure drop and therefore energy loss in order to operate properly. Another example was the use of an asymmetric population of resin. Such a resin can result in spread out exit profiles and more dilute, voluminous products which require additional energy to concentrate, handle or transport. This paper argues that efficiencies related to energy use are hindered whenever a less symmetric approach is used to force a symmetric outcome. If the symmetry hypothesis is correct, using turbulence for mixing is inefficient (this is not to disregard the usefulness of turbulence at the smallest scales, where heat and mass transfer occur or where conditions require the use of turbulence for practical reasons).

In addition to high energy use, turbulence can result in inhomogeneous process conditions. For example, in a large impeller mixed reactor, temperature, concentrations and reaction conditions may be quite variable throughout the volume.
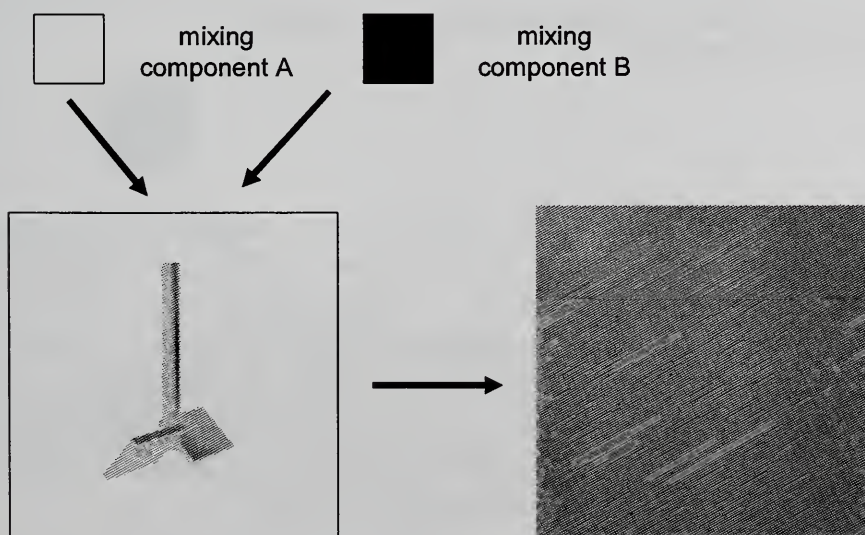
Figure 17. Asymmetric, energy intensive impeller mixing results in a product symmetric to the distribution of A and B.

The symmetry hypothesis of this paper suggests that addition of symmetries to the mixing process will be beneficial. Figure 18 illustrates a favorable increase in the symmetry of mixing components A and B *prior* to employing an asymmetric process element (turbulence). For example, this could represent an increase in symmetry occurring by some presently undefined procedure *before* turning on an impeller.

Consider how little subsequent turbulent energy would be required if the initial conditions were, for example, Symmetry 3. In fact, Symmetry 4 is the completely mixed state with no turbulence used at all. Of course, the problem is how to actually approximate these states.

Symmetry 1   Symmetry 2   Symmetry 3   Symmetry 4
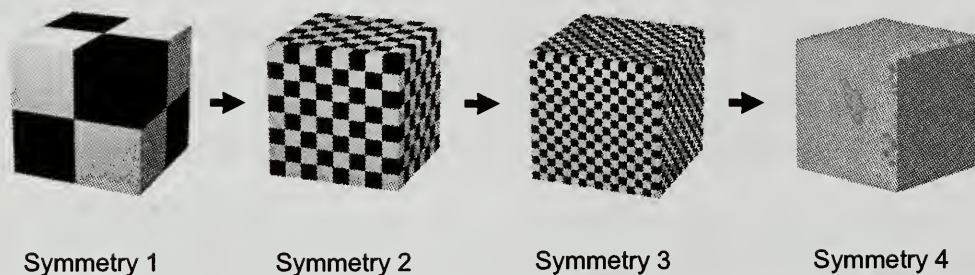
Figure 18. Initial conditions for mixing components A and B *prior* to using turbulence for final mixing.

A solution discussed in previous papers is to use structures referred to as "turbulence alternatives"(3,4,5). These are low pressure drop symmetric fractals. They substitute symmetric scaling and distribution for the asymmetric scaling and distribution of turbulence. Figure 19 illustrates a fractal appropriate for increasing the symmetry of a mixture prior to final mixing. For example, the device can be used to distribute fluid A into fluid B while at the same time spinning as a low energy impeller (Note that the checkerboard pattern in this and all following examples is just a qualitative indication of an increase in symmetry - no such patterns could actually form).
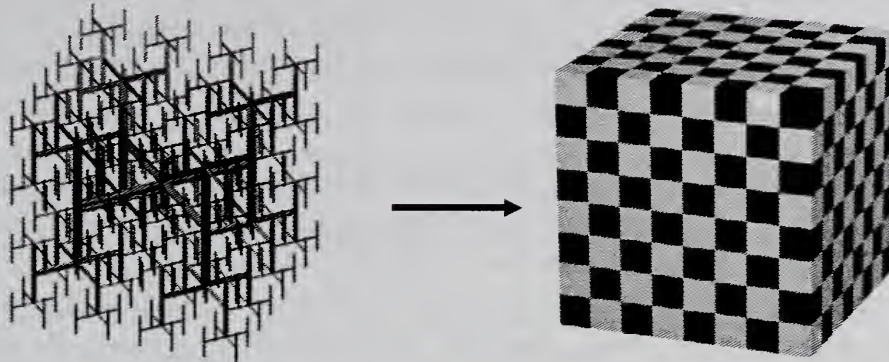
Figure 19. A low pressure drop fractal used as a fluid distributor and impeller can provide a more symmetric mixture prior to energy addition. Therefore overall energy required is reduced.

Figure 20 illustrates a second method to force symmetry on a mixing system. In this case, two fluids are simultaneously scaled prior to mixing by the use of offset fractals. Note that both fluids are "mixed" before they contact one another. By adding symmetry to both fluids in the system, the asymmetry of turbulence is avoided and mixing is more efficient with respect to energy use. The symmetry eliminates the non-uniformities observed with impeller driven mixing. These non-uniformities can include concentration, temperature, turbulent intensity etc.

mixing
component A
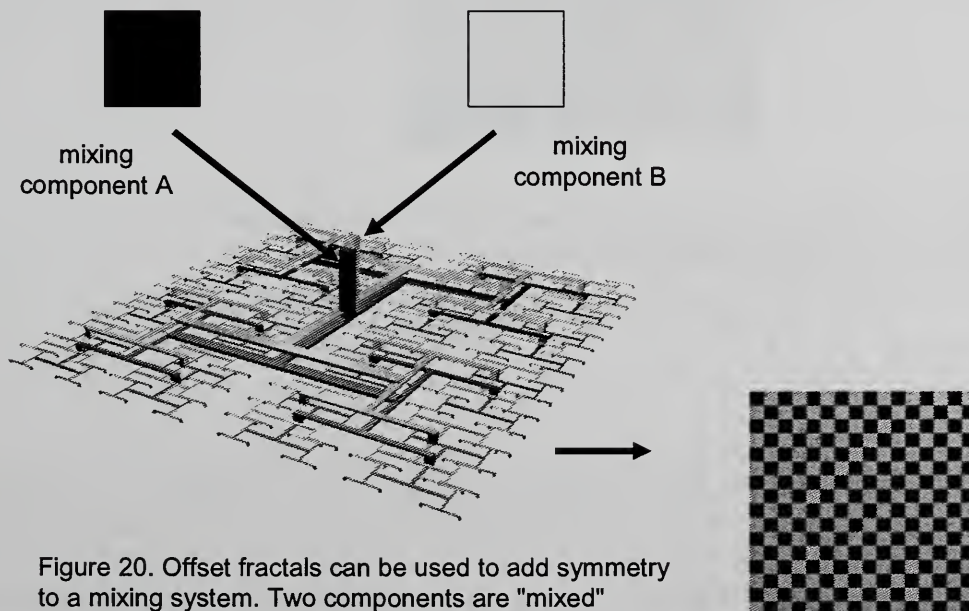
mixing
component B

Figure 20. Offset fractals can be used to add symmetry to a mixing system. Two components are "mixed" before they contact one another.

Also, because of the overall system symmetry, a desired change in mixing/reactor conditions can be realized nearly instantaneously as opposed to an asymmetric system where system inertia and inhomogeniety may require a significant amount of time before a change is observed everywhere in a vessel. This means the fractal mixer/reactor is more symmetric over time - a change in conditions, such as temperature or concentration occurs everywhere at the same time.

Finally, it was previously indicated that faster mixing is obtained with turbulent methods by increasing the energy input per unit time (if the same device is used). For example, impeller RPM is increased or components are shaken more violently. How is the mixing rate increased with a fractal mixer? This is accomplished by increasing the symmetry of the device. For example, by using smaller and smaller structure allowing closer approximation of a completely symmetric checkerboard. Figure 21 is a close-up photograph of an offset fractal mixer/reactor.
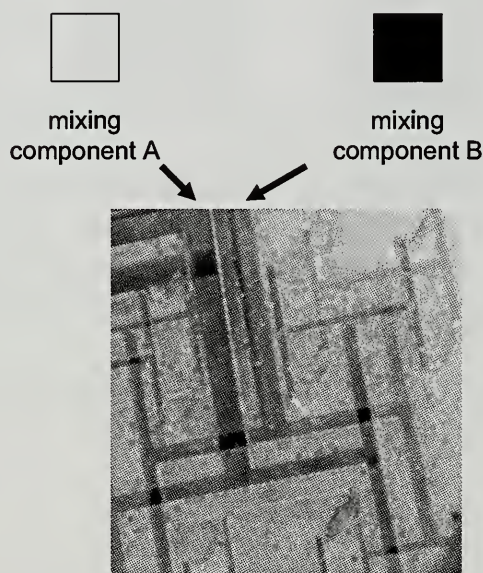
Figure 21. Close-up photograph of the final channels of an offset fractal mixer/reactor (Amalgamated Research Inc.).

Although fractals were used in these mixing examples, they are only one category of structure for forcing symmetry on an otherwise turbulent process. It is suggested that the usual way of using large scale turbulence does not contradict the symmetry/efficiency hypothesis. It is perhaps more likely that large scale turbulence is very often used in an unnecessary, inefficient manner.

Asymmetric processes

There are processes which require an asymmetric outcome. For these cases, the process forcing should address the asymmetry. An interesting example is clarification (separation of solids from liquids) because this process can gain efficiency by both increasing the symmetry of elements for which a symmetric outcome is desired and by increasing the asymmetry for elements for which an asymmetric outcome is desired. Figure 22 illustrates a continuous introduction of clarifier feed wherein the incoming fluid forms a completely symmetric surface with respect to velocity (no turbulence disturbance).

If this symmetry could be practically implemented, it would lead to much smaller equipment operating under rapid separating conditions. However, for a clarifier, it is also necessary that the subsequent final outcome be asymmetric. Solids move to the bottom of the clarifier and clear liquid moves to the top. This final solids/liquid distribution is asymmetric compared to the starting homogeneous solid/liquid feed material.

Where an asymmetry is required, forcing asymmetries is appropriate. Note that an asymmetry is not desired in velocities of the fluid (turbulence and its associated chaotic accoutrements are not desired) but rather between the liquid/solid components. The liquid-solids difference in density provides an asymmetry which will lead to separation in a gravitational field. The asymmetry can be increased by a settling aid which further reduces the liquid-solids symmetry of the solution (Figure 23).

Summarizing some of the main process elements for improved clarification in terms of symmetry:

1. Create a symmetric velocity condition for the fluid introduction.
2. Use settling aid to increase asymmetry in the liquid-solids mixture.
3. Use gravity to completely eliminate the liquid-solids symmetry.

Goal: Approximate the introduction of a symmetric fluid surface with velocity = 0
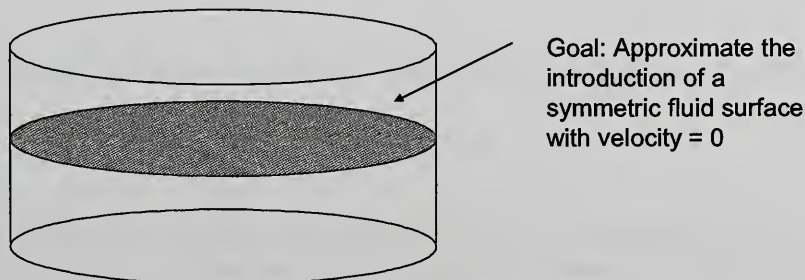
Figure 22. Clarifier operation can gain efficiency by increasing the velocity symmetry of the fluid introduction. Additional symmetry can be added by including symmetric collection of liquid and solids.
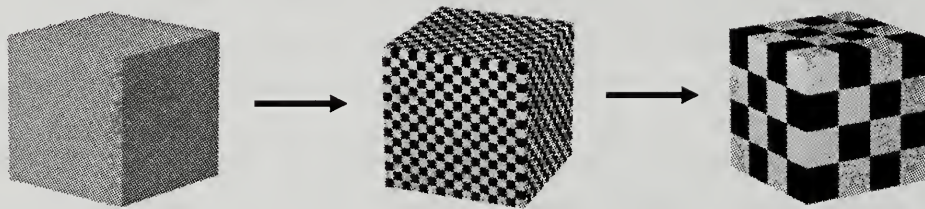
Figure 23. Adding a settling aid to a clarifier solution increases solution liquid-solids asymmetry and increases the efficiency of settling.

Processes which contain an asymmetric element include separations, such as clarification and chromatography. Essentially all the process elements in chromatography require a symmetric outcome, many the same as in the ion exchange example. Only the differential migration of components through the chromatographic media is a desired asymmetry. If the asymmetry of the media with respect to component affinities can be increased or by some other manner magnify the asymmetry, the chromatography process will be more efficient.

Conclusions

A commonality has been determined which applies to diverse aspects of process efficiency. This commonality is symmetry. As a working hypothesis, this paper proposes that increasing symmetries or asymmetries is a general manner of addressing a wide variety of disparate process efficiencies.

It is suggested that a process be separated into individual elements and these elements be evaluated for two cases:

Case 1
The desired outcome of the process element is symmetric. In this case, process efficiency is improved by increasing the symmetry of the process element.

Case 2
The desired outcome of the process element is asymmetric. In this case, the process efficiency is improved by increasing the asymmetry of the process element.

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Beverage Standards and Testing and Trends in Sugar Quality for Beverages

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Abstract

High quality supplies of beet and cane sugar are critical to the success of the beverage industry. An extensive set of specifications has been developed in an effort to ensure that contaminants do not affect the quality of our beverage products. These specifications will be discussed. In recent years, problems have developed with some sugar supplies that are within existing specifications in that they lead to development of malodors in beverage products. The results of our research aimed at the identification of malodorants and pro-malodorants will be discussed.

Introduction

Supplies of high quality sugar are critical to the success of the beverage industry. And to ensure that the sugar supplies meet The Coca-Cola Company quality needs, we have evolved an extensive set of specifications. These specifications are discussed in this paper. As the sugar industry has evolved over time, manufacturing processes have changed in effort to reduce cost while maintaining quality. Some of these process changes have created the opportunity for introduction of novel contaminants into the final refined product. At The Coca-Cola Company, we are particularly concerned about contaminants that lead to beverage off-tastes or off-odors. We have initiated a comprehensive research program to develop an understanding of such contaminants and to develop analytical methods for their quantification. Our efforts on this program are very much a work-in-progress and the results we discuss in this paper are preliminary. We expect that our sugar specifications may change in the future based on the findings of our research.

It is of course true that the principal contribution of sugar to beverages is sweet taste. And, while there are many other sweeteners that are used in beverages, sugar remains the standard for sweet taste quality. How is it that sugar initiates this magical perception that is so universally liked by consumers, young and old? Yes, it excites our taste buds! But beyond that, the level of scientific understanding of what sugar does in our taste buds has not been at all clear. Very recently, Elliot Adler and associates at Senomyx (La Jolla, CA) have identified the receptor in the cells of human taste buds that recognizes sugar, presumably in a classical lock-and-key manner, and initiates the sweet taste response.[1] In looking to the future, it would not be a surprise to see this sweetener receptor employed in analytical methods for assessment of sugar quality.

Specifications

The Coca-Cola Company has developed specifications for sugar in effort to ensure that sugar supplies purchased for use in beverages meet our quality needs (Table 1). In this paper, I am specifically focused on aspects of quality that relate to "off" taste and "off" odor. The Coca-Cola Company's specifications for sugar quality are given below. As relates to "off" taste and "off" odor, Specification Nos. 2 (Taste), 3 (Odor) and 14 (Odor after Acidification) are the areas of focus for this paper. In each case, the specification is dependent on a human sensory assessment of taste or odor. In our experience, such testing is quite *subjective* and prone to error. For this reason, we are very interested in the development of *objective* analytical methods for these specifications.

The Granulated Sucrose must meet the specifications in the United States Food Chemicals Codex (FCC) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) standards, including some specific requirements of The Coca-Cola Company. Upon receipt, it should comply with the following plus any other standards defined by local food regulations, consistent with its intended use.

Progress in a Study of the Malodorous Contaminants of Beet Sugar

On a weight basis, sugar is the major ingredient in sugar-sweetened beverages, with the exception, of course, of water. And, as a part of a program targeted at ensuring the quality of the beverage products of The Coca-Cola Company, we initiated a project aimed at the identification of any malodorants or pro-malodorants in beet sugar. Our objectives are not limited to the identification of such materials but extend to the development of analytical methods for them and to the revision of Company specifications where necessary to ensure their control.

Hydrogen Sulfide Generation Potential. In our work with beverages, we have found hydrogen sulfide (H_2S) to be one of the most offensive malodorants. And we have found that H_2S levels in contaminated beverages increase from the time they are formulated thus suggesting the presence of sulfurous compounds which decompose under acidic conditions to generate H_2S. For this rationale, we explored approaches to determine the H_2S Generation Potential (HGP) of sugar samples. We began by analyzing the headspace above solutions of beet sugar in dilute phosphoric acid by gas chromatography (GC) with sulfur chemiluminescence (SC) detection.[2] As expected, we found H_2S to be quite common in these analyses and, in addition, found low

levels of carbonyl sulfide, carbon disulfide, methanethiol, dimethylsulfide, dimethyldisulfide and dimethyltrisulfide as well as two unidentified sulfur compounds. However, since H_2S is a much more potent malodorant than the other sulfur compounds, we focused on it. Our headspace GC method clearly demonstrates the evolution of H_2S from sugar samples. However, in its current state of development, it is limited in at least 3 ways:

1. We found detection of H_2S by GC to be rather capricious; sometimes it was easily detected and other times not detected at all. We believe this to be due to the high reactivity of H_2S toward metal surfaces and, even though precautions were taken to ensure that all surfaces were silanized by the Silcosteel process, many times H_2S, inexplicably, could not be detected.

2. Since the partition coefficient of H_2S between the headspace and the phosphoric acid / sugar solution is not known, total H_2S could not be directly determined.

3. The time required for conversion of all sulfurous sugar contaminants to H_2S could not be easily determined since H_2S appeared to be degrading to unknown breakdown products under the conditions of its analysis.

As a result of the limitations of the GC method, we developed an ion selective electrode (ISE) method for sulfide ion, which is quantitatively generated on passage of H_2S into alkali.[3] In this method, as in the GC method, sugar samples are heated in dilute H_3PO_4. However, here, the H_2S is swept from the reaction vessel into a caustic solution. The H_2S contents of the sugar samples are then calculated based on the sulfide ion concentration determined by ISE.

In order to evaluate the relative utilities of the GC and ISE methods for H_2S analyses, 14 beet sugar samples were analyzed by both methods. The results are summarized in Table 2.

It is noteworthy that, while the HGP results obtained by the GC method are only semi-quantitative, they compare reasonably well with the findings by ISE analysis which should quantitatively reflect the potential of sugar samples to release H_2S. The ISE method for determination of the HGP of sugar samples is an acceptable method. However, it still is a method requiring about a half-day per sample and a skilled analyst. And therefore there is a need for a simplified and rapid method that could be employed by manufacturing plant personnel. We also wish to note that while the human threshold for detection of H_2S in air is <1ppb (v/v)[4] and, while it can be calculated that any of the beet sugar samples in Table 2 could release H_2S into a beverage headspace substantially in excess of 1ppb, we have not observed H_2S malodor from beet sugar samples with HGP in this low $\mu g/kg$ range. The HGP range at which H_2S malodor becomes a problem is under investigation.

Table 1. The Coca-Cola Company specifications for granular sucrose.

For this attribute...	Meet this specification...	Refer to...
1. Appearance	White crystals or crystalline powder, with no more than 4 black specks per 500g, or must pass test.	SM-PR-465, or equivalent method
2. Taste	Typically sweet and free from foreign tastes.	Taste, Odor, and Appearance – Nutritive Sweeteners, (SM-PR-420)
3. Odor	Free from foreign odors.	SM-PR-420
4. Assay (Purity)	Not less than 99.9% w/w: polarization or, calculated as 100% sucrose minus ash, minus moisture, minus invert.	ICUMSA
5. Ash	Not more than 0.015% w/w (conductivity).	ICUMSA
6. Color	Preferably less than 35, but not more than 50 Reference Basis Units (RBU) [or ICUMSA Units (IU) equivalents]. Material higher than 35 RBU/IU must be specifically approved by The Coca-Cola Company.	ICUMSA
7. Floc Potential	Must pass test.	Floc Potential Tests, (SM-PR-270)
8. Copper	Not more than 1.5 mg/kg.	ICUMSA
9. Arsenic (as As)	Not more than 1.0 mg/kg (FCC).	ICUMSA/FCC
10. Invert Sugar	Not more than 0.1% w/w (FCC).	FCC
11. Lead	Not more than 0.5 mg/kg (FCC/JECFA).	ICUMSA/AOAC/ FCC
12. Microbiological:		
Total Bacteria	Less than 200 cfu per 10 grams.	ICUMSA
Yeast	Less than 10 cfu per 10 grams.	
Mold	Less than 10 cfu per 10 grams.	
13. Moisture	Not more than 0.06% w/w.	ICUMSA
14. Odor After Acidification	Free from objectionable odor.	Odor After Acidification – Nutritive Sweeteners, (SM-PR-310)
15. Quaternary Ammonium Compounds	Not more than 2 mg/kg in sugar refined by any process that uses Quaternary Ammonium Compounds (QAC).	SM-PR-470, Quaternary Ammonium Compounds
16. Screen Size	Not more than 7.5% finer than 65 mesh when screened for 10 minutes, or must pass test.	ICUMSA, or equivalent method
17. Sediment:	Not more than 7 mg/kg gravimetric insolubles.	Sediment – Nutritive Sweeteners, (SM-PR-415)
18. Sulfur Dioxide	Less than 6.0 mg/kg.	AOAC/ICUMSA
19. Turbidity	None in 50% w/w solution, or must pass test.	SM-PR-420, or equivalent method

Table 2. Analysis of 14 beet sugar samples for HGP by two analytical methods.

Sample No.	HGP by GC/SC (μg/kg Sugar)	HGP by ISE (μg/kg Sugar)
RSM-19	1.7	2.4
RSM-20	2.4	3.3
RSM-21	3.2	3.0
RSM-22	3.1	1.6
RSM-23	4.8	6.3
RSM-24	2.5	4.1
RSM-25	2.7	2.4
RSM-26	1.7	1.5
RSM-27	2.4	1.6
RSM-28	3.8	3.0
RSM-29	3.1	1.0
RSM-30	3.5	2.5
RSM-55	1.0	2.5
RSM-65	4.6	4.8

Hydrogen Sulfide Origin on Acidification of Beet Sugar. As has been discussed above, we have evidence that beet sugar samples undergo reactions in acid media to release H_2S. But, we have no knowledge as to its origin. We believe it to be due to the acid hydrolysis of organic sulfur compounds to release H_2S but we do not know their identities. In an effort to determine what they are, we made extracts of sugar samples and analyzed them by GC/HRMS.[5] This led to the identification of methylisothiocyanate (MITC). At the time, we had no idea as to the origin of MITC. On searching the literature, however, we learned that MITC is a breakdown product of the biocide metham-sodium used in the beet sugar industry.[6] Metham-sodium breaks down by two pathways as illustrated in Scheme 1.

Scheme 1. The Hydrolytic Degradation of Metham-sodium.

$$CH_3-NH-\overset{\overset{\displaystyle S}{\parallel}}{C}-S-Na \xrightarrow{H_3O^+} CH_3-N=C=S \ + \ S=C=S$$

Metham-Sodium   MITC

$$\downarrow H_3O^+$$

$$CH_3NH_2 \ + \ H_2S \ + \ CO_2$$
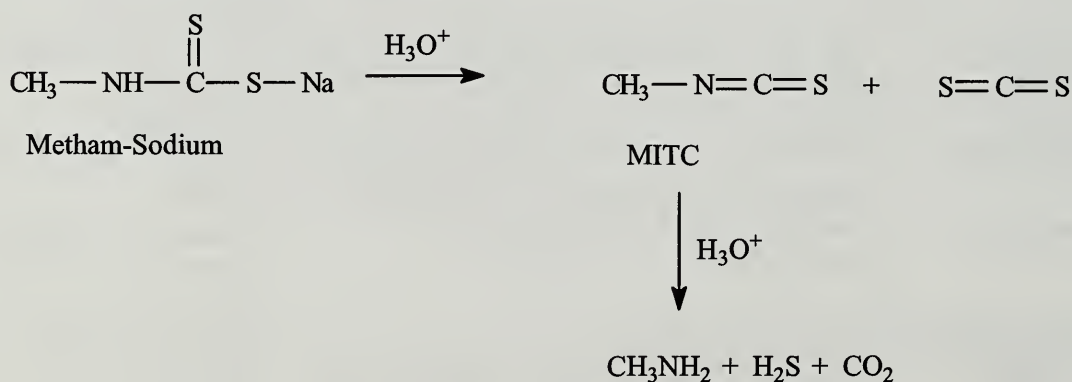
We also learned of a class of natural compounds known as glucosinolates that degrade to isothiocyanates.[7] The glucosinolate precursor of MITC is a natural product, as is MITC itself, and, in fact, MITC has been described in the literature as a natural biocide.[8] The MITC glucosinolate precursor and its conversion to MITC are illustrated in Scheme 2.

Scheme 2. MITC Formation from its Glucosinolate Precursor.
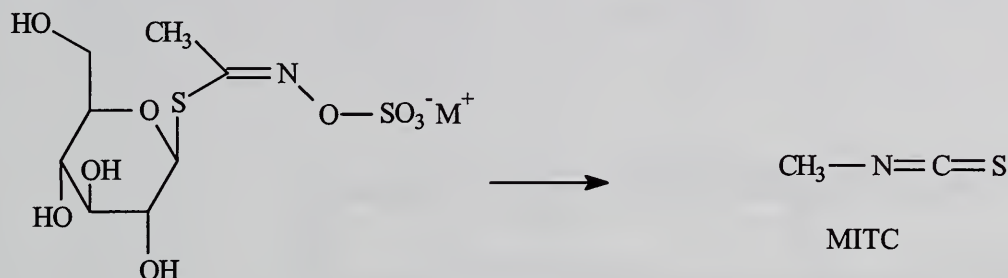
The biogenesis of glucosinolates has been the subject of considerable investigation and it is known that they form in a number of plant species from natural amino acids. And thus a series of natural glucosinolates derived from most of the amino acid building blocks of protein have been identified. These glucosinolates also undergo degradation to produce isothiocyanates just as the alanine-derived glucosinolate breaks down to produce MITC. We reasoned that if the MITC we have identified in beet sugar extracts is glucosinolate derived, these extracts should also contain isothiocyanates corresponding to other amino acids. However, MITC is the only isothiocyanate we have been able to identify in beet sugar extracts. This finding argues for MITC's origin being metham-sodium, probably following its use as a biocide in the sugar manufacturing process.

Since it is known that isothiocyanates undergo reaction with alcohols to produce thiocarbamate condensation products and since we demonstrated the presence of MITC in beet sugar samples, we were concerned that these sugar samples may contain substantial amounts of sugar/MITC condensation products as illustrated in Scheme 3. An intramolecular equivalent to this reaction has been described where methyl 2-deoxy-2-isothiocyanato-α-D-glucopyranoside undergoes intramolecular condensation with a vicinal hydroxyl group to produce a cyclic thiocarbamate.[9] In order to explore the possibility that MITC-containing sugar samples may contain sugar/MITC condensation products, we studied the reaction of MITC with sugar by NMR.[10] However, we found no evidence for the reaction of MITC with sugar to produce thiocarbamate condensation products.

It also seemed to us that if the origin of MITC in beet sugar samples was the naturally-occuring, alanine-derived glucosinolate shown in Scheme 2, then MITC should be present as a trace contaminant in all beet sugar samples. To explore this possibility, we analyzed for MITC by GC/HRMS the same series of 14 beet sugar samples that were analyzed above for HGP. The results of this analysis, summarized in Table 3, show that MITC is not an omnipresent beet sugar contaminant. Thus we conclude that MITC, when present as a sugar contaminant, is most likely metham-sodium biocide derived.

Scheme 3. Hypothetical Reaction of MITC with Sugar.
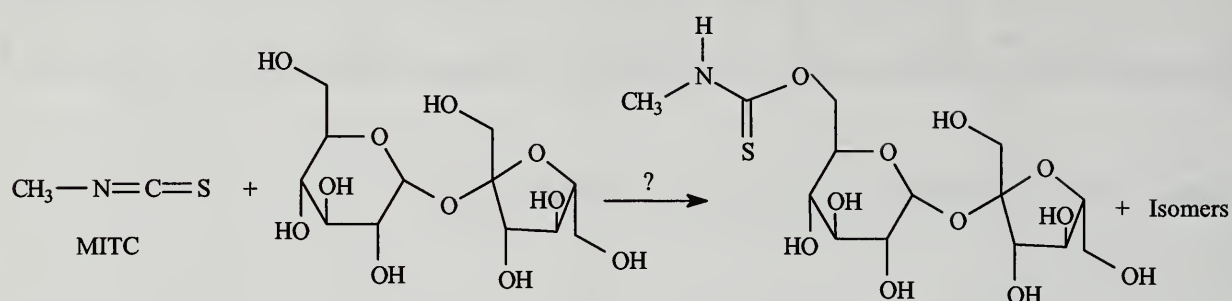
Table 3. Analysis of 14 beet sugar samples for MITC.

Sample No.	MITC (μg/kg Sugar)
RSM-19	0.0
RSM-20	0.0
RSM-21	0.0
RSM-22	0.0
RSM-23	0.0
RSM-24	0.0
RSM-25	0.0
RSM-26	0.0
RSM-27	0.0
RSM-28	0.0
RSM-29	0.0
RSM-30	10.0
RSM-55	0.0
RSM-65	0.0

So, it is clear that some of the H_2S formed on acidification of beet sugar samples is derived from hydrolysis of MITC. However, as is also clear from comparison of the data in Tables 2 and 3, there must be additional H_2S sources beyond MITC. We assume that these additional H_2S sources must be organic sulfur compounds. In order to estimate the levels of unknown sulfur-containing organic compounds present in beet sugar samples, we analyzed the samples for inorganic sulfur compounds known to be present, then analyzed the samples for total sulfur content and, by difference, calculated the percentage of total sulfur content which may be due to organic sulfur compounds. This analysis was carried out on the same set of beet sugar samples analyzed above and on which data is provided in Tables 2 and 3. These samples were analyzed for sulfite and sulfate by ion chromatography.[11] In this work, we made the observation that sulfite is not stable to the conditions of analysis. We found that it is oxidized to sulfate. However, we found that this oxidation process could be nearly halted by the addition of EDTA to the sample vials. Presumably, EDTA chelates metal ions (e.g., Fe^{3+}/Fe^{2+}) which promote an autoxidation reaction. Our results are summarized in Table 4 and an ion chromatogram showing the sulfite and sulfate analytes is illustrated in Figure 1.

Table 4. Analysis of 14 Beet Sugar Samples for Sulfite and Sulfate.

Sample No.	Concentration (mg/kg)	
	Sulfite	Sulfate
RSM-19	2.65	5.42
RSM-20	3.43	7.43
RSM-21	3.27	8.32
RSM-22	5.07	11.05
RSM-23	3.32	5.12
RSM-24	0.00	4.43
RSM-25	5.72	3.61
RSM-26	3.37	6.63
RSM-27	2.27	12.57
RSM-28	1.91	7.89
RSM-29	0.03	0.99
RSM-30	0.91	7.29
RSM-55	0.05	1.39
RSM-65	0.03	4.94
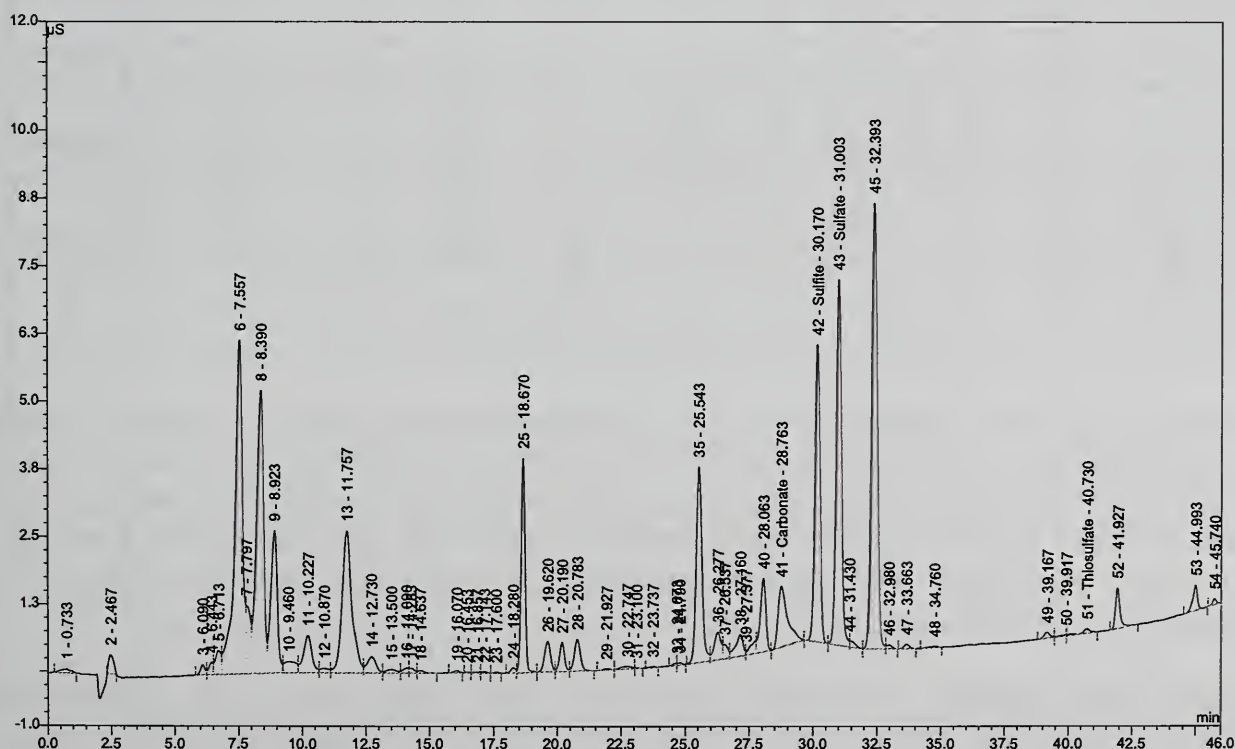
Figure 1. An Ion Chromatogram of a Typical Beet Sugar Sample. Sulfite Ion appears with $t_R = 30.17$ min and Sulfate Ion appears with $t_R = 31.00$ min.

The same beet sugar samples as were used for the analysis in Table 4, were also analyzed for total sulfur content. The levels of sulfur present in these sugar samples are close to the minimum quantifiable level by the combustion / microcoulometric titration which we employed.12 The sulfite and sulfate results summarized in Table 4 were used to calculate a value for inorganic sulfur content of each sample and then the organic sulfur content of each sample was determined by difference. The results for total sulfur, inorganic sulfur and organic sulfur that were obtained in this way are summarized in Table 5.

Table 5. Estimation of the organic sulfur content of 14 beet sugar samples.

Sample No.	Concentration (mg/kg)		Sulfur Percentage Composition		
	Inorganic Sulfur (SO_3^{2-} and SO_4^{2-})	Total Sulfur (Replicates)	Inorganic (Replicate 1)	Inorganic (Replicate 2)	Organic (100– Ave Inorganic)
RSM-19	2.9	9.6 / 5.0	30	57	57
RSM-20	3.8	10.4 / 7.9	37	49	57
RSM-21	4.1	6.8 / 6.8	60	60	40
RSM-22	5.7	12.0 / 8.5	48	67	42
RSM-23	3.0	6.3 / 6.0	48	51	50
RSM-26	3.6	10.5 / 5.1	34	70	48
RSM-27	5.1	__ / 6.0	__	85	15
RSM-28	3.4	__ / 5.5	__	62	38
RSM-24	1.5	4.2 / 3.0	35	49	58
RSM-25	3.5	6.4 / 5.5	55	64	41
RSM-29	0.3	__ / 3.1		11	89
RSM-30	2.8	__ / 4.0	__	70	30
RSM-55	0.5	__ / __	__	__	__
RSM-65	1.7	__ / __	__	__	__

This indirect method for organic sulfur compounds suggests that significant levels of organic sulfur compounds may be present in the beet sugar samples analyzed. Of course it is also possible that these samples contain inorganic sulfur compounds in addition to metal salts of sulfite and sulfate. If this is the case, the levels of organic compounds will be lower than estimated here. Additional work will be necessary to specifically identify organic sulfur compounds present in these samples.

Short-Chain Fatty Acids. It is well known that short-chain fatty acids (SCFAs) are contaminants of beet sugar and that, among them, butyric acid and isovaleric acid are particularly problematic malodorants due to their odorant potencies. For this rationale, we analyzed the same set of beet sugar samples as used throughout this study for SCFAs. Ion chromatography was used for this analysis.13 Interestingly, we found that the concentrations of several of the SCFAs decreased as a function of time before analysis of the samples prepared for ion chromatographic analysis. However, we were able to solve this problem by addition of sodium azide to the sample vials; apparently several of the SCFAs are undergoing microbial fermentation in the

absence of the sodium azide. Our results are summarized in Table 6 and a typical ion chromatogram is illustrated in Figure 2.

Table 6. Analysis of 14 Beet Sugar Samples for 13 SCFAs.

Sample No.	Short-Chain Fatty Acid Concentration (mg/kg) in Sugar*												
	FA	AA	PA	BA	iBA	VA	iVA	CA	iCA	HA	LA	OA	pGA
RSM-19	4.03	10.34	1.84	0.54	0.63	0.18	0.00	0.16	0.92	3.52	11.52	1.86	7.14
RSM-20	2.19	9.15	0.51	0.21	0.20	0.14	0.22	0.00	0.82	3.15	17.52	1.11	9.34
RSM-21	2.37	8.02	1.30	0.24	0.24	0.15	0.21	0.00	1.53	3.72	12.27	6.41	8.34
RSM-22	3.32	9.64	1.19	0.38	0.25	0.17	0.00	0.00	2.19	5.66	13.17	0.31	12.47
RSM-23	1.43	6.34	0.78	0.21	0.13	0.15	0.22	0.00	0.96	2.85	12.08	0.85	8.34
RSM-24	1.85	6.46	0.58	0.32	0.31	0.00	0.25	0.00	<0.11	3.13	24.58	0.70	9.95
RSM-25	2.16	4.53	0.73	1.16	0.45	0.13	0.21	0.00	1.74	4.50	11.09	3.91	14.58
RSM-26	2.15	6.51	0.78	0.51	0.20	0.21	0.21	0.00	0.48	5.01	8.57	1.88	9.04
RSM-27	3.56	9.47	1.30	0.32	0.30	0.17	0.00	0.00	1.48	5.63	15.22	1.63	15.05
RSM-28	1.74	4.93	0.47	0.19	0.18	0.00	0.21	0.00	0.34	3.41	10.55	0.28	9.48
RSM-29	2.18	5.40	0.17	0.25	0.53	0.00	0.00	0.00	<0.11	0.69	16.13	0.30	3.29
RSM-30	1.32	1.88	0.50	0.25	0.33	0.00	0.00	0.00	0.16	1.98	4.05	1.35	4.11
RSM-55	0.44	1.38	0.65	0.19	0.19	0.00	0.00	0.00	0.88	1.51	2.73	0.16	3.95
RSM-65	2.20	3.66	0.64	0.35	0.39	0.00	0.00	0.00	0.49	2.80	14.18	1.69	13.02

*FA = Formic Acid, AA = Acetic Acid, PA = Propionic Acid, BA = Butyric Acid, iBA = Isobutyric Acid, VA = Valeric Acid, iVA = Isovaleric Acid, CA = Caproic Acid, iCA = Isocaproic Acid, HA = Heptanoic Acid, LA = Lactic Acid, OA = Oxalic Acid and pGA = Pyroglutamic Acid.

On inspection of the results in Table 6, it can easily be seen that butyric acid and isovaleric acid are not major contaminants in any of the 14 beet sugar samples. But yet, because of their odor potencies, they are the most problematic SCFA contaminants. We have not yet determined the specific levels of butyric and isovaleric acids at which they become malodorant problems once

the sugar is used in beverages. However, data we have gathered from the literature on the odors of these SCFAs and their thresholds is summarized in Table 7.
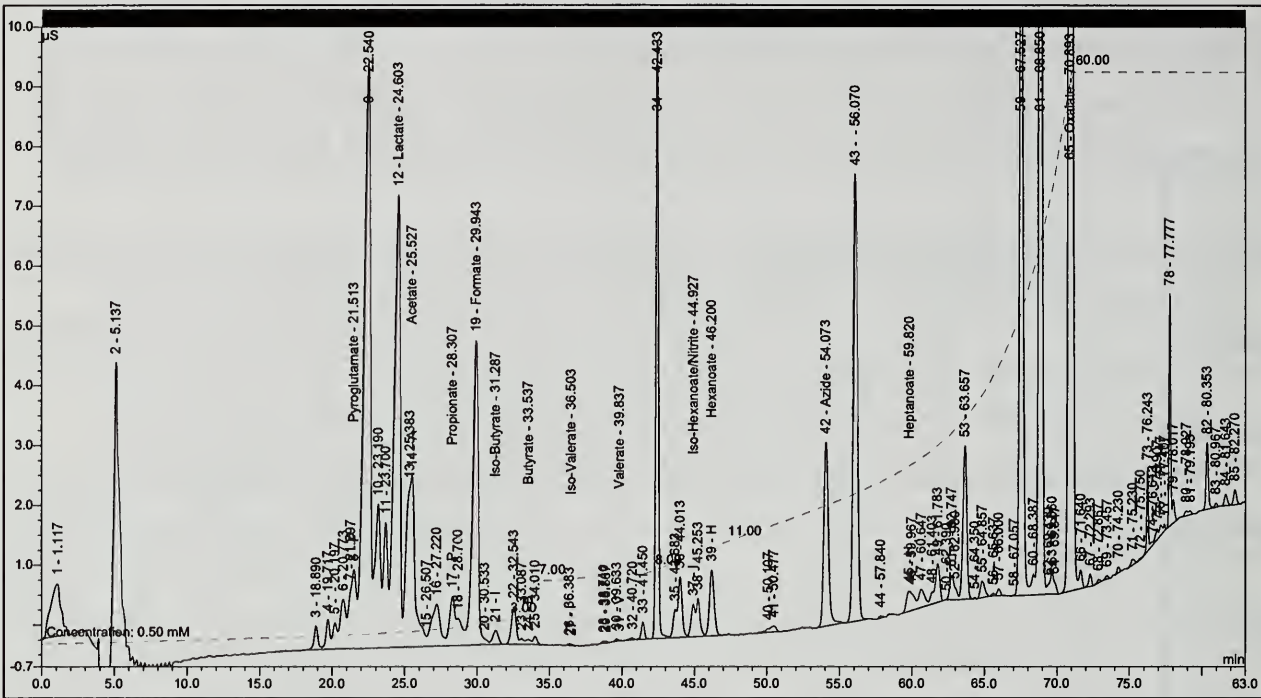
Figure 2. An Ion Chromatogram of a Typical Beet Sugar Sample with SCFAs Identified.

Table 7. Odor Character and Olfactory Thresholds for SCFAs Found in Beet Sugar.

SCFA	Olfactory Threshold (ppm in air)	Odor Character
Formic Acid	46-3650	Pungent
Acetic Acid	10-522	Acidic
Propionic Acid	5-10	Pungent
Butyric Acid	0.24-4.8	Rancid Butter
Isobutyric Acid	0.01-9.5	Cheesy
Valeric Acid	0.94-3.0	Sweaty or Cheesy
Isovaleric Acid	0.19-2.8	Pungent, Cheesy or Silage-like
Caproic Acid	0.93-10	Sweaty, Fatty, Cheesy
Isocaproic Acid	?	?
Heptanoic Acid	0.64-10.4	Sweaty, Rancid or Fatty
Lactic Acid	?	?
Oxalic Acid	?	?
Pyroglutamic Acid	?	?

Terpenoid Alcohols, Pyrazines and Other Beet Sugar Malodorants. Other potential malodorous beet sugar contaminants include the musty/earthy terpenoid alcohols geosmin, which has been detected in beet sugar,[14] and 2-methyl-isoborneol,[15] which has been identified in the wash water used in sugar beet processing. In addition, the 4 burnt/coffee pyrazines shown in Figure 3 have been identified in sugar beets[16] and in liquid sugar products.[17] We have not yet developed methods for the analysis of these compounds or analyzed our set of beet sugar samples for them. This work is in progress.

Geosmin   2-Methyl-Isoborneol
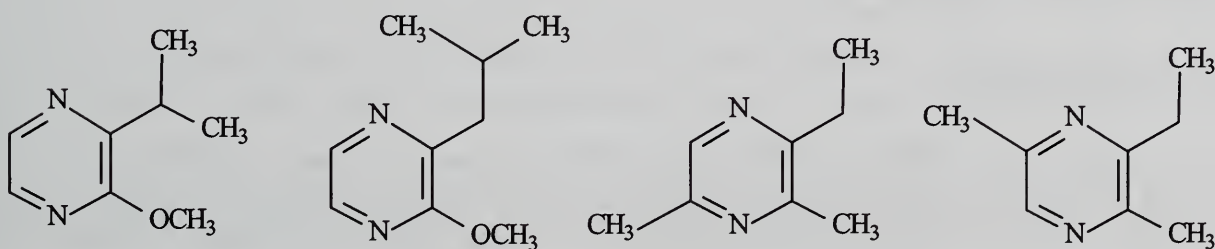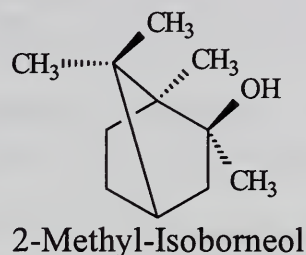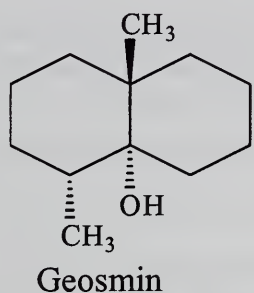
Figure 3. Terpenoid Alcohol and Pyrazine Malodorants Known to Contaminate Beet Sugar.

Conclusions

1. Beet sugar is a potential contributor to H_2S malodor in beverages.

2. The probable source of beet-sugar-derived H_2S malodor is metham-sodium, a dithiocarbamate biocide employed in the beet sugar industry.

3. Reliable analysis of H_2S at low levels is a challenge.

Future Work

As emphasized in the Introduction of this paper, this is a work-in-progress. We have just begun to develop and validate reliable methods for the beet sugar malodorants which concern us. Specific challenges we hope to address in the months to come include:

1. Development of a reliable and simple method for the determination of H_2S Generation Potential for sugar samples.

2. Determination of the maximum allowable H_2S Generation Potential for sugar samples based on odor perception in sugar-sweetened beverage products.

3. Optimization of analytical methods for total sulfur and organic sulfur content of sugar samples.

4. Identification of the organic sulfur compounds present in sugar samples.

5. Determination of the maximum allowable levels of Short Chain Fatty Acids for sugar samples based on odor perception in sugar-sweetened beverage products

6. Identification of analytical methods for other malodorants (e.g., geosmin methylisoborneol, pyrazines, etc.) present in sugar.

7. Determination of the maximum allowable levels of terpenoid alcohol, pyrazine and other non-sulfurous malodorants for sugar samples based on odor perception in sugar-sweetened beverage products.

Acknowledgements

Notes and References

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2. The Sample Headspace Analysis was carried out with a Tekmar Model 7050 Headspace Analyzer (HA) interfaced to a Hewlett Packard Model 5890 Gas Chromatograph and a Sievers Model 355 Sulfur Chemiluminesence Detector (SCD). The analysis was carried out on a 60m x 0.53mm 7μm Restek MXT-1 column. The column pressure was 5.0 psig with the injection port operated in the splitless mode. The injection port temperature was set at 150°C. The analysis was carried out with a temperature program (35°C for 2 min, then increased to 200°C at 15°C/min and then held at 200°C for 2 min). All chromatographic components contacted by the sample were Silcosteel treated to minimize H_2S degradation. The SCD furnace was set at 804°C, with oxygen and hydrogen flows set at 8 and 100mL/min, respectively. Sugar samples (3.00g) were combined with 10 mL of 1M H_3PO_4 in 22 mL vials designed for the Tekmar HA. Thiophene (3.3ng; 20μL of 164.6μg/L thiophene in MeOH) was added to each sample as an

internal standard and H_2S analytical results are reported as $\mu g/kg$ H_2S in the sugar samples but are really thiophene equivalents based the thiophene response factor and H_2S peak area. The sample vials were heated at 80°C for 60 min, then pressurized at 17.5 psig for 0.25 min with a pressure equilibration time of 0.25 min. The 0.50 mL sample loop was filled for 0.03 min with a loop equilibration time of 0.10 min. The injection time was 1.00 min. The sample loop temperature was 150°C.

3. Sulfide ion concentration was determined with an Orion Model 9616 sulfide ion selective electrode (ISE) and an Orion Model 720A pH meter. The ISE was calibrated with $Na_2S \cdot 9H_2O$ solutions prepared in an antioxidant buffer (AB = 0.2M EDTA and 0.2M Ascorbic Acid in 2M NaOH). H_2S content of sugar samples was determined by dissolution of 100.0g samples of sugar in 204 mL of H_3PO_4 (27 mL of 85% (w/w) H_3PO_4 diluted with 177 mL H_2O) and heating at 85°C for 3 h while sparging the reaction vessel with nitrogen with the effluent carried into a trap containing 50 mL of AB. At the conclusion of the 3 h reaction time, the sulfide ion concentration was determined by the ISE. In an experiment designed to test the method, Na_2S was used as a control; ISE analysis of the sulfide ion trapped showed an 84% recovery of sulfide. In control experiments on compounds known to generate H_2S on acid hydrolysis, methylisothiocyanate (MITC) and metham sodium were added to the acid reaction vessel in place of sugar. In both cases, the conversions to H_2S were less than 10% of the theoretical yields.

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5. The analysis was carried out by GC/MS (Agilent 6890 / Micromass Autospec Ultima). The GC analysis was carried out in the Splitless Mode with an Injector Temperature of 250°C, a Flow Rate of 1.00 mL/min, an Injection Volume of 1.00 μL, a Temperature Gradient (35°C for 4 min, 4°C/min to 60°C, 60°C for 0 min, 10°C/min to 250°C and 250°C for 20 min) and on a J&W DB5 Column (30 m x 0.32 mm i.d. x 1 μm film thickness). The MS Detector was set for EI Positive Ion Detection with a Source Temperature of 250°C, a Filament Current of 400 μA, the Detector set at 300 Volts and the Resolution at 10,000. The PFK peak at 68.9952 was used as a Mass Lock. Under these conditions, the MITC Molecular Ion at 72.9986 exhibited a relative intensity of 100%, the MITC (M-1) peak at 71.9908, a relative intensity of 80% and the MITC (M+2) peak for the [34]S isotope at 74.9941, a relative intensity of 4%. The samples for GC/MS analysis were prepared by extraction of a solution of 25.0 g of the sugar sample and 0.5 g NaCl in 100 mL water with CH_2Cl_2 (3 X 15 mL). The combined extracts were concentrated to ~0.5 mL, diluted to 1.00 mL and analyzed. Ethylisothiocyanate was used as an internal standard in these analyses.

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10. The reactivity of MITC toward sucrose was evaluated by ^{1}H NMR on a 400 MHz Bruker NMR instrument. MITC was first evaluated in D_2O and found to be quite stable; a single ^{1}H absorption at 3.14δ was observed which did not change after 5 days at ambient temperature. Two new singlet absorptions did appear, however, at 2.46 and 2.67δ after 30h at 60°C. In order to assess the reactivity of MITC toward sucrose, 41 bmg (0.56 mmole) MITC was combined with 200 mg (0.58 mmole) of sucrose in 5.5 mL D_2O in an nmr tube. No loss of MITC was observed after 17 h at ambient temperature and after a further 40 h at 40°C; however the same MITC degradation products generated by MITC alone (i.e., with ^{1}H absorptions at 2.46 and 2.67δ) were observed.

11. The Inorganic Sulfur Compound Analysis was conducted with a Dionex 600-DX Ion Chromatograph equipped with Autosampler, EG40 Eluent Generator and Conductivity Detector. The analysis was carried out on a Dionex AS11-HC (250 x 2.0 mm i.d.)] Column at 30°C. A Dionex AMMS-III (2 mm i.d.) Suppressor Module was used, operating in the chemical suppression mode (w/ 50mM H_2SO_4, at flow rate of ca. 5.0 mL/min). The non-degassed, purified water eluent was automatically programmed at three different gradients with KOH solution from 1.0 to 60.0 mM at a flow rate of 0.38 mL/min at ca. 2,300 psi, using the Eluent Generator and PeakNet© Program. The Eluent Generator has 9 standard gradients (Gradient Nos. 1-9), where Gradient No. 1 rises rapidly initially and is concave downward, Gradient No. 5 is linear and Gradient No. 9 rises slowly initially and is concave upward. In this method, the analysis was begun with Gradient No. 7 [1.0 – 10 mM KOH (0 – 10 min)], followed by a second phase at Gradient No. 7 [10 – 60 mM KOH (10 – 43 min)] and a third phase at Gradient No. 5 [60 mM KOH (43 – 46 min)]. The sugar sample (2.000 g) and 20 µL 100 mg/L EDTA were combined and diluted with degassed purified water to 10 mL in a volumetric flask. The injection volume was 100 µL. The gradient program requires 1 h 9 min (including a 15-min pre-run equilibration) per analysis.

12. The Total Sulfur Analysis was conducted with a Mitsubishi TOX-100 Total Sulfur Analyzer. In this method, a 50-100mg sample of sugar is accurately weighed and transferred to a sample boat that is then transferred to a pyrolysis tube in which the sample is combusted in an oxygen atmosphere. Under these conditions, all sulfur present is converted to sulfur dioxide which is then carried to a titration cell in which it is titrated coulometrically against triiodide ion. The analyses were carried out by Galbraith Laboratories (Knoxville, TN).

13. The SCFA Analysis was conducted with a Dionex 600-DX Ion Chromatograph equipped with Autosampler, EG40 Eluent Generator and Conductivity Detector. The analysis was carried out using two Dionex AS11-HC (250 x 2.0 mm i.d.) Columns connected in series at 39°C. A

Dionex AMMS-III (2 mm i.d.) Suppressor Module was used, operating in the chemical suppression mode (w/ 50mM H_2SO_4, at flow rate of ca. 5.0 mL/min). The non-degassed, purified water eluent was gradient programmed at six different gradients with KOH solution from 0.5 to 60.0 mM at a flow rate of 0.32 mL/min at ca. 2,600 psi, using the Eluent Generator and PeakNet[©] Program. The Eluent Generator has 9 standard gradients (Gradient Nos. 1-9), where Gradient No. 1 rises rapidly initially and is concave downward, Gradient No. 5 is linear and Gradient No. 9 rises slowly initially and is concave upward. In this method, the analysis was begun with Gradient No. 7 [0.5 – 3.0 mM KOH (0 – 32 min)], followed by a second phase at Gradient No. 5 [3.0 – 7.0 mM KOH (32 – 34 min)], a third phase at Gradient No. 5 [7.0 – 8.0 mM KOH (34 – 42 min)], a fourth phase at Gradient No. 5 [8.0 – 11.0 mM KOH (42 – 47 min)], a fifth phase at Gradient No. 8 [11.0 – 60.0 mM KOH (47 – 71 min)] and finally a sixth phase at Gradient No. 5 [60.0 mM KOH (71 – 83 min)]. The sugar sample (2.000 g), 10 μL of 500 mg/L NaN_3 and 20 μL 100 mg/L EDTA were combined and diluted to 10 mL with degassed purified water in a volumetric flask. The injection volume was 250 μL. The gradient program requires 1 h 50 min (including a 15-min pre-run equilibration) per analysis.

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Ethanol Production in Brazil:
A Successful History

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Abstract

The Brazilian experience, regarding the use of ethanol as automotive fuel, began in 1923 by the addition of ethanol to gasoline. Since then, the proportion of ethanol to gasoline has changed throughout the years mainly due to fluctuations in sugar and alcohol prices in the international market. In 1975, the Federal Government launched the "Proalcool" program to reduce the country's dependence on oil importations that some years before had reached 80 percent of its oil needs. Nowadays, ethanol, sugar and bioelectricity have opened new perspectives and transformed Brazilian mills and distilleries into bioindustries for food and energy. The aim of this paper is to show the positive impact of the Brazilian ethanol program on the economy, engines, energy, employment and environment, as well as, price stability making sugar and ethanol, the role of bioelectricity and the forecast to next years.

Ethanol Production: Impact in Brazil

Since the beginning of the "Proalcool" program, the country has saved more than 140 billion dollars with imported oil and interests, while sugarcane production increased from 91 to 406 million tons per year in 2005. Nowadays, sugarcane is cultivated by more than 70,000 farmers in an area of 6 million hectares corresponding to less than 3% of the total cultivating area in Brazil. In the same period, ethanol production jumped from 0.56 to 16.8 million m^3/year while sugar production increased from 6 to 27.5 million tons/year. The development of new cane varieties, better soil practices, control of plagues and diseases, as well as new harvest systems, increased the field productivity from 53 tons per hectare/year in 1977 to 90 tons per hectare/year in 2005. During this period, the percentage of sugar in sugar cane was increased from 9.5% to 14% while the sugar extraction was improved from 88% to 96-98%. Moreover, by measuring and monitoring industrial parameters of alcohol fermentation processes, it was possible to improve the fermentation yield from 75-78% in 1977 to 90-92% in 2005. Today, there are 370 distilleries in the country, and the sugar and alcohol sectors employ more than one million people.

Some years ago, automobile industries developed new engines and launched flex-fuel vehicles, which are designed to operate on pure ethanol, pure gasoline or any combination of both. They have become quite popular in Brazil because of the high gasoline prices. Nowadays, there are more than 30,000 gas stations to attend to 16 million cars powered by gasoline blended with 20 to 25% of ethanol as well as more than 2 million cars running on hydrated ethanol only or in any combination of gasoline and ethanol (flex-fuel vehicles).

Concerning the environmental impact, the main benefit of ethanol use as a fuel has been the reduction of carbon dioxide, heavy metals and other toxic pollutant emissions in large cities like Sao Paulo and Rio de Janeiro. Ethanol is a clean fuel, and the production process is almost entirely sustainable because carbonic gas is recycled by sugarcane without releasing fossil CO_2 into the atmosphere in the same proportion as gasoline or diesel oil. Considering the entire production chain, there is a positive balance resulting from ethanol production processes because for each energy unit consumed, another nine units of energy are generated (Ometto, 2004). Furthermore, sugar cane removes CO_2 from the atmosphere, which is immobilized in organic matter in the soil. Because of its renewable process of production the distilleries have been considered potential candidates to receive carbon credits from the Kyoto Protocol.

Fermentation Process: Main Characteristics

Ethanol production in Brazil is done during a continuous sugar cane harvest season of 200-230 days. These industrial processes are based on large-scale fermentations of sugar cane juice, molasses or a mix of both in different proportions. Alcoholic fermentations have been carried out in very large fermentors with capacity from 0.4 to 2.0 million liters each. Despite differences among distilleries, there are two main fermentation systems: feed-batch and continuous fermentation (Amorim and Lopes, 2005). Approximately, 75% of the distilleries have used the feed-batch system. Moreover, the industrial fermentations are well characterized by their high yeast cell concentrations (8-17%), very short fermentation times (6-10 hours) and alcoholic concentrations between 6-11% (v/v). After the end of each fermentation cycle, yeast cells are centrifuged off from the beer and treated with diluted sulfuric acid for $1 - 2$ hours at pH 2.0-2.5 to kill bacteria. After this treatment, the yeast cells are returnd to fermentation vats to start a new fermentation cycle. Yeast strains such as PE-2 and CAT-1 (Fermentec) or BG-1 and SA-1 (Copersucar) were selected from industrial fermentations by karyotyping techniques and evaluated in the laboratory for many years (Wheals, *et al.*, 1999). These strains are adapted to industrial processes and show high resistance to stress conditions. In 2006, more than 190 distilleries began the fermentation process with selected yeasts strains.

Price Stability

Nowadays, the prices of sugar and ethanol have a strong mutual influence in Brazil. When the sugar prices in the international market are more profitable than ethanol, more sugar cane is milled to sugar production, while the molasses is sent to fermentation and ethanol production. In the last 30 years the percentage of cane sent to sugar production has been variable (from 26.5% to 86.3%) but has been around 50% in the last 5 years. Another aspect to be considered is international oil prices. When oil prices are above US$40, ethanol is competitive in Brazil (Carvalho and Oliveira, 2006). Ethanol production cost in Brazil is US$1.00 per gallon, while

the cost of ethanol production from corn (dry milling process) has been very close - US$1.05 per gallon (Shapouri, *et al.*, 2006).

Bioelectricity

Many Brazilian distilleries are based not only on sugar and ethanol but also on bioelectricity production. Sugarcane leaves and bagasse can be burned to produce steam and electrical power for the industry as well as to be sold to electrical companies. Because sugar and ethanol are made during the dry season, it is advantageous to produce electricity during the driest period in the year, when water reservoirs are low. Sometimes there are problems with the electricity supply from hydroelectric plants during the dry season, but distilleries can supply this energy during critical periods.

Forecasting

Brazil has met appropriate conditions to develop its ethanol industry including economic and political stability, a large market and distribution nets for sugar, ethanol and bioelectricity, crop area, sugar cane varieties, know-how in alcoholic fermentation and specialized technical staff. Moreover, Brazil has all necessary technology and industries to build new mills and distilleries.

There are three important centers of plant/design builders in the State of Sao Paulo: Piracicaba, Ribeirao Preto and Sertaozinho. They supply projects and equipment such as samplers, milling tandems, diffusers, decanters, evaporators, centrifuges, distillation columns and any other equipment needed to build a mill.

Because of these favorable conditions, the forecast for the next six years is to expand sugar cane areas on to pasture land (Goldenberg, 2006). The total sugar cane area in Brazil will reach 8 million ha by 2012 (Biagi, 2006) while more new 89 mills will begin to produce ethanol and sugar. Brazilian production will reach 560 million tons of sugar cane, 27.3 billion liters of ethanol, 35 million tons of sugar and 6,000 MW of bioelectricity (Biagi, 2006).

Then, we have been witnesses that many mills and distilleries are building a successful history looking to new horizons based on ethanol, sugar and bioelectricity production.

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Current Status of Biotechnology in Sugarbeet and Sugarcane

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ABSTRACT

One simple definition for plant biotechnology in sugar crops can be techniques that use living cells of organisms or parts of those cells with a practical goal of producing plants and services that benefit sugarcane and sugarbeet industries. This paper describes the current use of biotechnology in cane and beet crops of the international sugar industry.

Improved sugarcane and sugarbeet varieties have traditionally been developed using conventional sexual methods of plant breeding. Technologies developed since 1980 can now be used to enhance existing breeding efforts by developing services to enhance sugar production and genetically engineered plants which are best referred to as "biotech plants". The immediate goals for biotech crop utilization would be to improve profitability, improve sugar quality, reduce the environmental impact of crop production, and develop alternate products from each crop. Specific objectives for biotech sugar crops include herbicide resistance; pest resistance (insects and diseases); higher sugar content; stress tolerance (freeze, drought, flood, salt); alternate products including pharmaceuticals, ethanol or other energy products; and other characteristics specific to each crop.

Experimental sugarcane varieties have been developed as biotech varieties and are currently being tested in several industries although no commercialization has moved forward to date. Biotech sugarbeets have cleared government regulations in the U.S. and commercialization is anticipated within the next few years. Numerous organizations have been involved in cane and beet industries to undertake the research and regulatory issues that need to be achieved in order to proceed with utilization of biotech crops on a commercial scale. The future for biotech in sugarbeets appears to be of considerable economic potential and as simple as other seed propagated crops since the distribution and sale of biotech material is easily controlled. The future for sugarcane also has great potential but is more complicated since it is a vegetatively propagated plant and biotech seed cane can be more difficult to manage. Herbicide tolerant

plants appear to be the most readily usable characteristic for both sugarcane and sugarbeet crops. Alternate product development, especially in sugarcane, with its high biomass potential, also seems particularly promising for the future. Marketing of sugar produced from biotech sugarbeet and sugarcane plants had been considered an issue in the past, but considerable progress has been made toward its acceptance in the market place.

Introduction

This paper discusses a subject of interest that is perhaps vital to the success of all sugar industries around the world. The subject matter concerns the science of biotechnology which describes the role of a body of methods and techniques that employ as tools the living cells of organisms or parts or products of those cells (such as, genes and enzymes). The word "biotechnology" consists of two parts: "bio" indicating life and living organisms and "technology" indicating the input of human art, knowledge and ability. Therefore we can further describe biotechnology as the use of organisms or parts of organisms to produce certain products. There have been products on the market for years that have been produced with the use of biotechnology: for example beer or yoghurt. Beer is produced with the help of yeast cells and yoghurt is made by two kinds of bacteria. Today, more and more products derived from biotechnology are brought onto the market: medicines, such as insulin for the treatment of diabetes, have been used for years, while others have only recently been introduced. Genetically modified food crops, such as corn and soybeans, have been grown internationally for years, generating some discussion about these products in only certain areas. Biotechnology affects all of our lives; therefore it is important that we know at least a little about the subject matter.

The key to economically viable crop production generally lies in varietal improvement. Conventional breeding is normally thought of as the means to improved varieties. But there are alternatives to conventional variety improvement methods and biotechnology provides us with some of those. This paper describes some of the potentials of biotechnology as well as some of the basic techniques involved. There are several organizations that have assisted with the development of biotech work. The level of sugarcane biotech work in each country varies considerably, and this paper describes that level in the major producing countries. The U.S. sugarbeet industry is well advanced in biotech development. The paper concludes with the future of biotechnology in sugar crops.

Crop Improvement: Conventional Breeding

It does not matter whether you are a grower, processor or refiner, and whether you are trying to make sugar or alcohol; the objective is the same--To make a profit! Making money is often done by increasing the volume of product produced through area increases or factory capacity. However, for the sugarcane or sugarbeet grower, volume increases can and often have involved raising the actual yield per unit area of land. Examples include the volume of sugar produced from the U.S. sugarbeet industry and the Louisiana sugarcane industries over time. Some of the increase in production was due to expansion of area harvested. However, as studies have shown, the increase from higher yielding varieties, both tons of crop and higher sugar per ton, have

contributed significantly. There are dramatic fluctuations from year to year as a result of weather or government programs but, over time, the trends are a positive reflection of improved varieties. For this reason, industries normally proclaim that improved varieties are the first step to improved profitability.

Improved varieties normally come about from extensive breeding programs that nearly every sugarcane and sugarbeet industry conducts for itself. The major difference between sugarcane and sugarbeet programs results from their difference in propagation methods. Sugarbeets are planted by true seed, while sugarcane seed consists of vegetative material (stalks and buds). As a result, international sugarcane breeding programs are conducted almost exclusively by government or industry funded programs. Production of one stalk of a new variety produces everything a grower needs to start the utilization of that variety. Thus there is little profit potential for a private company to involve itself with sugarcane breeding. Sugarbeets, on the other hand, rely on true seed for propagation. As a result, government or industry programs often produce breeding lines (elite parents) while private companies produce improved varieties for commercial production.

Breeding programs in both crops normally begin with the crossing of elite parents that have been identified as outstanding in numerous characteristics and contain genes for traits that could provide superior progeny when they recombine. However, to select for these combinations of traits takes an extensive selection program that in most industries involves many program stages. A typical breeding program begins with parental selection and crossing, followed by several stages of testing on small plots (often at government or private experiment stations) and concludes with large scale testing on farmer's properties under "real world" conditions. After all of the tests are concluded, superior varieties can be "officially" released to an industry. In many selection programs, superior varieties are identified from within the selection stages to begin the next cycle of breeding as elite parents in a recurrent selection system.

As a result of the many stages involved, breeding programs can take a very long time, normally a dozen or more years from the time of crossing to potential release. This involves hundreds of thousands of plants and many research plots, numerous research workers, and normally requires several cycles to see significant improvement in most traits. A tremendous amount of effort, manpower, and money is required to achieve varietal improvement.

To add further discouragement, when one considers the amount of effort that goes into a breeding program, the percentage of varieties that are ultimately released to an industry is very, very small compared to the number of plants that initiated each cycle of breeding. A lot of time is wasted on varieties that are eventually discarded from the program.

Crop Improvement: Biotechnology

Crick and Watson are credited with the discovery of the "secret of life" in 1953 which we now know as the double helix of deoxyribonucleic acid or DNA. While an important discovery, it is within the last thirty years that techniques have been developed which can assist in the modification of the plant's genetic makeup and assist in developing improved varieties. These techniques involve a molecular approach and deal with the contents of the cells of the plant

rather than breeding the plant itself. This work has often been called genetic engineering or genetic modification.

In the broad term and not necessarily concerning plant improvement, when the public thinks of genetic engineering, they often think of scientists, normally wearing white coats, and sometimes producing products that later might be identified as a "monster". Unfortunately some of the public has translated these thoughts to the current efforts at using genetic modification to produce food products they might think of as "Frankenstein foods" or "monster foods". Lots of information has been published about genetically engineered plants and animals and the good or bad concerning these products has long been debated. One thing is certain, and that is the consumer or public has the right to say what they want about the use of genetically engineered food crops. And for that reason, lots of surveys and studies have shown us that it would be better if we referred to products that are derived from genetic engineering as simply "biotech varieties" rather than using the words, genetically engineered, transformed or modified. Throughout this paper the word "biotech" is used to describe the work that we have in the past called genetically engineered or genetically modified.

The sugar crop breeder has a goal in mind whether they use conventional techniques or biotech variety development. Traditional plant breeding attempts to move a desired gene or genes from a donor plant by sexual crossing of two parents. It is hoped that the desired gene(s) will recombine into a new plant variety that has the best of both parents. Unfortunately, not only is the desired gene(s) transferred but other genes are dragged along as well. And some of the desired genes from either parent may also have been lost. Therefore, traditional plant breeding can be thought of as a very "hit or miss" technique in terms of having the right combination of genes in the progeny.

Conventional breeding has worked well for a hundred years because the lack of precision has been compensated through the use of large numbers of progeny. Now it is time to look at newer technologies that might be able to assist conventional breeding programs and reduce the size and cost of these programs. In typical biotech variety development, the desired gene from a donor organism is transferred into a variety that has already been proven as acceptable. Since unwanted genes are not dragged into the new plant, a great deal of work can be saved. However, there are still tests involved to make sure that the chromosomal location of the new transferred gene doesn't cause the improved variety to react differently. Testing is still required but at least the testing of the extreme numbers of varieties that eventually will get discarded as in a conventional breeding program is reduced.

Reasons why sugar industries might want to use biotech varieties on their farms or in their factories can be identified. The most important objective any business person would have is an incentive for profit. Other reasons might be that sugar quality could be improved, environmental impact could be reduced, and perhaps alternate products could be produced. But economic viability is probably the most important to the businessman. However, it might be that based on other issues to be discussed later in this paper, the order of priorities should be reestablished.

Biotechnology Potential

Lots of research has already been undertaken to identify goals that might be achieved by using biotech varieties. Genes for herbicide resistance have been identified and are currently being tested. These genes make the biotech plant resistant or tolerant to that herbicide and the grower could control weeds more effectively and less expensively. Additionally, the negative impact of using various herbicides could be reduced by concentrating on one pesticide that would be relatively safe. For these reasons, herbicide resistance is usually discussed as a major objective of biotech work.

Disease and insect resistance are also exciting possibilities. Controlling these pests with no or limited use of pesticides would reduce production costs and certainly have a positive impact on the environment. Numerous genes have been identified or developed that give plants resistance to many bacterial, fungal and viral diseases. Some of these donor genes occur in sugar crops, others in various plant species and still others from the animal kingdom.

Probably one of the most encouraging goals would be research that demonstrates that higher sugar content could be achieved over that which conventional breeding has been able to provide. More sugar in each stalk or beet easily converts to more profit for the grower and for the processor.

Tolerance to the many stresses that sugarcane and sugarbeet producers and processors face could be extremely important to economic viability. Traits like tolerance to drought, salt, flooded or waterlogged soils, and cold or freezing conditions could be important to various industries. Control of flowering in sugarcane could be an important objective for some industries. And, probably one of the most dynamic and potentially industry changing objectives would be the development of alternate products. Common pharmaceuticals, cancer fighting drugs, proteins, and other chemicals that could be potentially made within the crop if the right gene were inserted into sugarcane and sugarbeet varieties could benefit mankind. Because sugarcane produces an extremely large amount of biomass per hectare, it has an advantage over most other row crops. Additionally, sugarcane has an existing harvest, transport and processing system that could accept the whole plant and process it if desired. Most other crops only harvest the grain or portions of the plant and leave the remainder in the field. Sugarcane already has a complete process that could take advantage of genes that might be considered for producing these alternate products. Sugarbeet, while lacking in the quantity of biomass also harvests the majority of the plant and could have similar advantages. The sky is probably the limit when it comes to alternate products that could be produced through biotechnology.

Biotechnology Techniques

There are five general steps involved with the process of crop genetic engineering. These steps are basically the same for all transformed plants which we refer to as biotech plants: Extract the DNA, clone a single gene, design the gene, transformation, and backcross breeding.

The steps in transformation have been well documented and numerous countries are now using them with many crop plants including sugarcane and sugarbeets. Sugarcane is one of the last

row crops to utilize biotechnology, but that is simply because of the complexity of sugarcane DNA as compared to corn, soybeans, rice or even humans.

Identification of genes is the normal starting point for engineering plants. Genes may already exist in a sugarcane or sugarbeet plant that can be transformed into other plants. However, many genes of interest occur in some other plant or animal. In sugarcane and sugarbeet, research within the U.S. has used genes from various organisms to develop the traits or characteristics needed. Once the gene is identified, a construct has to be developed which is comparable to a bullet that will be shot into the recipient plant cells. The bullet is composed of not only the gene of interest but a promoter gene to turn it on and terminator gene to turn it off. The target plants then need to be prepared to accept the bullet. That usually requires using something close to single cells of the recipient plant. This is accomplished in the laboratory using well documented tissue culture techniques. The transformation process can then be undertaken by any of several different techniques. One method inserts the construct into bacteria and allows the bacteria to carry the DNA into the sugarcane or sugarbeet cell. In essence, the bacteria infect the recipient cells with the new DNA which contains the gene of interest. Another procedure commonly used is a ballistic approach. Initially, gene guns used a 22 caliber bullet to inject the DNA into target cells. Modern gene guns use a stream of gas to propel the DNA into target cells. After the transformation process has been accomplished, the cells that have received the DNA must be selected from those that did not accept the donor material. This is usually accomplished using some easily identifiable or selectable marker that is included with the construct. The scientist removes the selected cells which are believed to carry the new DNA and begins the process of turning them back into sugarcane or sugarbeet plants. By using the proper growing conditions and appropriate media to grow these cells, they can once again develop leaves and roots and become a complete plant. However, the selection isn't complete because not all cells that contain the donated DNA actually have the genetic material inserted into a place on the chromosome that can accept it and express the gene of interest. Therefore, continued selection must be undertaken to insure that the transformed plants actually carry the new DNA alongside its existing genetic material. This usually requires greenhouse and field testing once the laboratory work is concluded. These tests are undertaken under highly secured conditions to insure that the transformed plants don't escape into commercial fields before the tests are complete and the biotech plant has been deregulated. The entire process is highly controlled to insure safety to the environment and to the public. Those plants that are shown to be transformed and can now carry a "biotech" label must be thoroughly tested to be sure that they still have other desired traits and to be sure they have acquired no bad traits. At this point, biotech plants could either be used commercially or as elite parents in a conventional breeding program.

Biotechnology: History of Sugar Crops and Current Status

During the late 1980s, a group of scientists realized that biotechnology was going to have great potential in sugarcane. It was already being used in other crops but hadn't moved to sugarcane because of the complex nature of sugarcane DNA. The Brazil, South Africa, Australia, Texas, Hawaii and Louisiana industries agreed to initiate what would become the International Consortium of Sugar Cane Biotechnology (ICSB). Initially it was thought that besides studying sugarcane DNA, the organization could also conduct transformation work which together would have yielded improved varieties. However, it was quickly realized that with different varieties in

each industry and the fact that each industry was interested in different traits, transformation was something that individual industries needed to undertake. Currently the Consortium consists of 19 members and continues to consider funding of various proposals. The work is generally in an effort to increase the understanding of the sugarcane genome and how science might assist in improving current varieties, thus improving profit.

Sugarbeet biotechnology has progressed with the assistance of the Beet Sugar Development Foundation (SBDF) and the American Sugar Beet Growers Association (ASBGA). Both organizations represent the interests of the U.S. sugarbeet industry. Working along with technology providers and seed companies, they have taken a transformed sugarbeet germplasm (event H7-1 which contains a gene making sugarbeet tolerant to the herbicide Roundup) and supported its commercialization. Event H7-1 was field tested beginning in 1998 and in 2003 Monsanto, as the technology provider, petitioned the United States Department of Agriculture for determination of non-regulated status. Similar requests were made to the U.S. Environmental Protection Agency and to the U.S. Food and Drug Administration for environmental, food and feed permits. All permits and deregulatory status have been granted in the U.S. and similar permits have been received from several foreign countries. There is currently a large scale demonstration of Roundup Ready® sugarbeets planted in four center pivot irrigation plots in the state of Idaho. Half of each center pivot is planted to a non biotech variety while the remaining half is planted to Roundup Ready® sugarbeets. Observations are being made on efficacy of weed control, associated costs, environmental issues and other factors.

The Sugar Industry Biotech Council (SIBC) has been formed and represents all aspects of the sugarbeet and sugarcane industries including seed companies and technology providers. This organization has worked to commercialize biotech sugar crops within the U.S. industry. The National Sugar Cane Research Council is a proposed organization that would represent the four U.S. sugarcane producing states and work toward commercialization of biotech sugarcane.

Transformation to achieve improved varieties is but one aspect of biotechnology. As part of the research, sugar crops will be studied and chromosome mapping will occur. Genes for specific traits will be identified and their location on specific chromosomes will be understood. This is analogous to drawing a road map of each chromosome in the plant describing where each gene is along this highway. Through biotechnology it is hoped that industries will find other useful genes that can be incorporated into the sugarcane or sugarbeet plant. It is also hoped that selection in traditional breeding program can be improved by associating the selection for desired traits with specific markers that can be more easily identified. Additionally, tests or assays that can be used to identify when a sugarcane or sugarbeet plant has a particular disease organism present is useful to help control plant pests. This is accomplished through the use of biotechnology. The science of biotechnology is not just transformation but consists of these techniques that can help keep industry members as efficient as possible in the production and processing of sugar, alcohol and other products. Ultimately, the goal continues to be maximizing profit and minimizing inputs.

A survey of various industries within the international sugarcane community indicates that there are no commercially grown biotech varieties of sugarcane. However, South Africa, Brazil, Australia, Colombia, Argentina, the U.S. and most recently India claim to have experimental

plots of biotech varieties. Other countries indicate they are working toward biotech sugarcane varieties. Biotech sugarbeet research is primarily being conducted within the U.S. When one considers the major producers of sugar, most are researching the commercialization of biotech crops. The main reason they have not progressed to commercial production thus far has generally been marketing concerns.

One of the issues that those in the U.S. feel strongly about is that biotech would be able to assist in the development of energy sources using renewable resources of sugarcane and sugarbeet. Energy production could be developed from biomass or from ethanol. Alternate energy products could also become a major issue.

Biotechnology: Concerns

The concerns of the buyers of sugar, consumers and industrial users have had impact on the progress of commercialization of biotech varieties of sugar crops. Other issues which have slowed some industries include the high costs and availability of the transformation process as well as the regulatory issues concerning biotech utilization.

There are environmental factors that some are concerned with including the escape of genes from biotech plants. Information has already been distributed by the U.S. sugarbeet industry to assure the public that utilization of biotech sugarbeet crops is safe. Sugarbeets are a biennial and as such flower in the second year of production. Commercial production of sugarbeets does not allow for the plant to flower since harvest occurs after the first year, eliminating the possibility of out-crossing of the biotech plants to other plant species. Other sugar industries need to address the environmental impact of biotech and make the facts available to the public.

Another concern deals with the nutritional and chemical aspects of the sugar itself. It has now been determined that sugar produced from biotech sugarbeets is substantially equivalent to that of sugar produced from non-biotech sugarbeets, meaning that the sugar is the same. Again, sugar industries need to continue to inform the public of the facts concerning biotech crops and the food products derived from them.

Biotechnology Future

In 2005, farmers around the world harvested biotech crops for the 10th consecutive year. Also, in 2005, the one billionth acre of biotech crops was harvested by farmers. Now that farmers have more than 10 years and nearly one billion acres of experience with biotech crops, it is a good opportunity to reflect on the proven economic and environmental benefits these crops provide, the solid track record of human health and environmental safety of biotech crops, and the promising future of benefits to come from new products currently in the research and development pipeline.

Biotech crops were planted and harvested in 1996 for the first time on a widespread, commercial basis. Since then, farmers have consistently harvested more biotech crops each year. In fact, the growth in acreage of biotech crops harvested has been more than 10% each year since 1996. Last year, farmers harvested 200 million biotech crop acres, a 20% increase over the previous

year and double digit growth in harvested acres was projected in 2005. Many farmers have made the choice to plant and harvest biotech crops. In fact, over 8 million farmers in 17 countries around the world harvested biotech crops in 2004. US farmers lead the way in the number of acres planted and reap the most productivity and environmental benefits as a result. However, biotech crops are not only grown by farmers in industrial countries. Fully 90% of the farmers choosing to plant and harvest biotech crops are located in developing countries where the benefits of these crops can make a substantial contribution to the alleviation of poverty. Globally, a majority (56%) of the soybeans produced are enhanced with biotech traits as is 28% of the cotton, 19% of the canola, and 14% of the world's corn.

Of the 200 million acres of biotech crops planted in 2004, the US ranked first in total planted acreage with nearly 118 million acres of biotech crops – mainly in soybeans, corn, cotton, and canola. The US is followed by Argentina (40 million acres), Canada (13 million acres), Brazil (12 million acres) and China (9 million acres). An additional nine countries planted biotech crops in 2004 and 14 countries planted more than 125,000 acres of biotech crops in 2004. For the first time, in 2004, the total growth in planted acreage of biotech crops was greater in low-to-medium income countries than higher income countries. Annual growth in planted acreage was greatest in India (+400%), Uruguay (+200%), Australia (+100%), Brazil (+66%), China (+32%), South Africa (+25%), Canada (+23%), Argentina (+17%), and the US (+11%). 90% of the number of farmers planting biotech crops are now located in low-to-medium income countries – solid evidence of the fact that biotech crops create benefits for farmers regardless of the relative size of their farms.

US farmers have readily adopted biotech crops, planting 118 million acres of biotech crops consisting primarily of soybeans, corn, cotton, and canola in 2004. Consumers around the world purchased nearly $28 billion of these biotech crops from US farmers during the 2003-04 crop year demonstrating their willingness to use biotech crops and ingredients. US farmers have rapidly adopted these products due to the significant economic and environmental benefits they create. A study summarizing the impacts for US farmers in 2003 alone was recently reported by the National Center for Food & Ag Policy (NCFAP): Increased net income for farmers of $1.9 billion across all biotech crops, increased net income from a combination of reduced production costs of $1.47 billion and increased yields of 5.3 billion pounds, creating an extra $409 million in revenue for farmers was noted. Farmers also decreased the application of pesticides by 46 million pounds and farmers decreased tillage in all crops where biotech traits were used.

According to a recent study, 63 countries are conducting plant biotechnology research and development across 57 different crops. More than half the research in plant biotechnology is taking place in developing countries. North America, Europe, China, Argentina, Brazil, South Africa, Australia and India are centers of influence that will lead the development of plant biotechnology in the future. China is emerging as an influential force in plant biotechnology and has invested hundreds of millions of dollars in biotech research, second only to the United States. India has more than 20 academic and research institutions involved in plant biotechnology research covering 16 different crops.

World wide, the regulatory process provides comprehensive oversight, ensuring safe, responsible and precise development of new biotechnology products. From discovery to field testing to

product development and even after commercial launch, regulatory agencies perpetually monitor, guide and counsel at every phase of the process. As a matter of fact, this has been validated in a European Commission report that summarizes 81 biotech research projects and concludes "…the use of more precise technology and greater regulatory scrutiny probably make them even safer than conventional plants and foods". For example, the experience with the Roundup Ready® trait now included in several crops substantiates the thoroughness of the regulatory process. Over a period of 15 years we have generated: Over 1000 Study Reports (10 yrs); Tens of thousands of Field Tests (14 yrs); 100k's Compositional Equivalence Analyses; Reviewed by 27 regulatory agencies in 13 countries.

Food and feed products containing ingredients derived from plant biotechnology crops will have a solid 10-year history of safe use and have been studied by top scientists and scientific organizations around the world for over 20 years. Several organizations, including those listed, have issued statements or official reports documenting the safety and benefits of plant biotech crops. Several billion meals containing biotechnology-derived foods or ingredients have been consumed by people around the world. As the FAO has pointed out, there is no reliable documentation of any food safety issues resulting from the introduction of genes, proteins, or traits through the use of plant biotechnology. Experience to date supports the conclusion that the regulatory process for plant biotechnology products has been successful and has resulted in the marketing of products that are at least as safe as conventionally bred equivalents.

Food and feed ingredients derived from biotech crops are currently in the food and feed supply around the world. Consumers are eating foods derived from biotech crops, as well as meat, milk and eggs from animals consuming feed ingredients derived from biotech crops. Market signals affect supply and demand globally.

These products, including starch and oil products we use every day in the global food chain are produced from biotech crops: Soybeans (oil, lecithin, protein isolate, protein concentrate, flour, and meal); Corn (oil, flour, high fructose syrup, and gluten feed); Canola (oil and meal); Cotton (oil, meal, whole seed, and hulls).

Food product categories that currently contain sugar along with biotech products include bakery/cereal, confections, dairy/ice cream, canned goods, and beverages.

The first generation of plant biotechnology has delivered many products creating real benefits. In the United States alone, more than 50 new agricultural products have completed all the federal regulatory requirements (from all relevant agencies) and may be sold commercially. Products with the most extensive commercial use are: herbicide tolerant crops such as Roundup Ready®, insect resistant crops such as Yieldgard® Corn Borer or Corn Rootworm, and virus resistant crops, primarily in fruits and vegetables.

Biotech sugarbeets are ready to move to commercial production within the next few years. Many sugarcane producing countries now have experimental biotech varieties. The use of biotech might be compared to a horse race. The good thing about a horse race is that there are several winners. So each sugar industry doesn't have to try to win the race outright (although the winner might get the most benefit); they just need to finish at least in the middle of the pack. In

this current horse race, sugarbeets are about to come across the finish line. Many industries seem to be headed down the back stretch and some can probably see the finish line ahead of them. Others are scattered around the track. There are some industries whose horse seems to be stuck in the starting gate and unfortunately there also seem to be industries that haven't even figured out which horse to ride. Biotech is a race, but one that many industries can finish and be a winner if they only get started.

Conclusion

Earlier in this paper, the objectives of biotech crop utilization were discussed. The highest priority factor concerned a profit incentive for producers of sugar. However, given the public concerns in Europe and the ever present potential of public distrust, industries should list factors that might benefit the public first. Production of medicines or products that could help the quality of life, reducing environmental impact and improving product quality are all items that should help agriculture industries market biotech crops to the people who buy food products. Therefore, while profit is important, satisfying the consumer should always be a major objective.

To conclude, many feel that biotech will be the next breakthrough that will allow industries to remain competitive in the international sugar community. Funding for biotech should not replace current research dollars, especially in breeding but rather should supplement that effort. While biotech research is costly, its potential could be valuable to all in the international sugar community.

Organic Sugar – Its Place in the World of Organic Products: Is There a Future?

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The term "Organic" means different things to different people. To a chemist, it refers to carbon-based molecules, but to a consumer of organic products it means much more. To an organic consumer, organic means the crop was produced without the use of man-made chemicals (herbicides, insecticides, chemical fertilizers, etc.). If it is a processed food, organic means using only organic and non-synthetic ingredients and chemicals in the process.

In an effort to standardize organic products, "certifying agencies" were formed to audit the growers and producers to insure standards were followed. Unfortunately, not all certifying agencies were able to agree on the same standards and still permit the food to be labeled as certified organic. Both the US and the EU have now established formal programs to bring more uniformity and definition to the whole organic movement. The USDA in the U.S. released in October 21, 2002, the organic standards 7CFR Part 205, also called the National Organic Program (NOP) which now governs the organic industry in the United States.

The NOP establishes:

1. Uniform standards for marketing, production, and processing of organically produced products.
2. Establishes a National List of substances approved for and prohibited from use in organic production and handling.
3. Establishes an accreditation program for certifying individuals and agencies that audit and certify organic production and handling.
4. Defines requirements for labeling products, certified organic, organic, or containing organic ingredients.
5. Provides for importation of organic agricultural products from foreign programs determined to have an equivalent organic program.

At this point in time the standards for agricultural production, processing, and production of foods in order to be labeled organic is more defined, but there are still differences in the standards between countries in the world. One example is in the EU, where sulphur dioxide is allowed in the production of organic sugar and in the U.S. where it is not allowed. There are

many other such anomalies and differences in international certification standards, causing some barriers to free world trade in organic products.

One of the tenets of the organic movement is the belief that the use of herbicides, insecticides, and chemical fertilizers to grow crops harms the environment. This can be a true statement if the use of these chemicals is abused. As a result, this has become an important part of the organic movement with the emphasis on a sustainable agriculture that does not harm the environment. There is much debate about this subject.

The global organic market for food and drink reached $23 billion in 2002, with North America overtaking Europe as the largest market for organic food and drink[1]. The organic market in the U.S. has seen an average growth of about 20% compounded yearly since 1990. Table 1 contains information starting in 1997 from the Organic Trade Association (OTA).

Table 1. Growth and penetration of the organic food market.

	Organic Food ($Mil)	Organic Food Growth	Total Food Sales ($Mil)	Organic Penetration % of Total Food
1997	$3,566	Na	$443,790	
1998	$4,372	19.8%	$454,140	0.8%
1999	$5,043	18.1%	$474,790	1.1%
2000	$6,104	21.0%	$498,380	1.2%
2001	$7,359	20.6%	$521,830	1.4%
2002	$8,624	17.2%	$530,612	1.6%
2003	$10,381	20.4%	$535,406	1.9%
2004	$11,904	14.6%	$544,141	2.19%
2005	$13,831	16.2%	$556,791	2.48%
2006	$16,000 (projected)			

Source: *Nutrition Business Journal estimates based on OTA's 2006 Manufacturer Survey, annual Nutrition Business Journal surveys of manufacturers, SPINS, and other sources.*

In 2005 the $13.8 billion in consumer sales of organic foods, when compared to $556.8 billion in total food sales, represents 2.5% of total U.S. food sales. The steady growth of the organic market in the U.S. demonstrates it is here to stay.

Little published information is available on just organic sugar and its growth. It should be safe to assume that the growth in organic sugar usage has followed the same relative growth rate the whole organic market has experienced since it is an important ingredient in many processed foods.

The majority of organic sugar is produced outside the U.S. and Europe. Most of the organic sugar is produced from organic sugar cane with only a small amount produced from organic sugar beets in England by British Sugar though this project, I believe, has now been discontinued. Florida Crystals of West Palm Beach, FL is the only organic sugar producer in the U.S. with about 4,000 acres of organic sugar cane and rice in production with an additional 900 acres planned to be put into production this year[2]. Brazil and Paraguay produce the most organic sugar in the world today.

Organic sugar (sucrose) must be produced from organic sugar cane or sugar beets. To obtain organic certification the fields must not have herbicides, insecticides, or chemical fertilizers applied for several years (three years for USDA certification while other countries may not have the same exact requirement). The yields can be less than traditional sugar cane production but work is being done using alternate crops in a rotation to improve the fertility and yields. There are also rules that govern harvest procedures and record keeping. All the above requirements make organic sugar cane more expensive to produce and growers need to realize a good return for the organic sugar cane to continue producing.

The main hurdle for organic sugar production is the lack of certified organic processing aids, in particular, flocculants that can be used to improve the separation (removal) of impurities during clarification. There are at least two organic flocculants reported to help in the clarification process; the seeds of the drumstick tree (*Moringa oleifera*) and the edible fruit of *Cordia myxa* in the literature[3]. The primary chemical used for clarification is milk of lime (a slurry of hydrated lime). Calcium hydroxide (CaOH) is not a chemical found in nature. It is man made, but it is the only chemical that works well in the sugar process and it is also the standard chemical used for clarification in traditional sugar production. Milk of lime adjusts the pH of the juice (keeping it stable and preventing sugar destruction) allowing the impurities that come in with the sugar cane to settle and be removed. Recently purists of the organic movement in the U.S. have tried to get many synthetic chemicals (even though they do not have a natural substitute) removed from the approved USDA list. Calcium hydroxide was one of many chemicals that were asked to be removed from the National List, and this would remove organic sugar from being certified as organic in the U.S. This move was defeated and calcium hydroxide is the only approved processing aid in the manufacture of crystalline organic sugar.

Organic sugar marketed in the world has a normal color range of 200-1200 ICUMSA units (45 max in conventional refined sugar). The lower the color, the more expensive it is to produce, due to additional boiling steps required (energy) to reduce the color in the final product. The normal range of sediment (insoluble matter) in organic sugar is 50 – 300 ppm (2 ppm max in conventional refined sugar). The higher sediment level is mostly due to not being able to use "normal" sugar processing flocculants and chemicals in clarification. Usually organic sugar is produced at a reduced rate in the same plant using the same equipment that produces traditional white sugar (at least this is the case in Brazil and Paraguay). Both growing organic sugar cane and producing organic sugar is more expensive than traditional sugar and therefore it is important to recover costs to remain viable. This can be a very delicate balance as the organic industry grows and large companies enter into the organic picture starting to make organic products for the first time but without a good understanding of the market dynamics of the organic industry.

I received permission from Wholesome Sweeteners, Inc. to provide the following information on organic sugar. An OTA survey on organic sugar was conducted and the results published in February 21, 2005. The OTA surveyed some of the largest users of organic sugar in the U.S. to obtain the reported trends. Twenty-one companies responded to the survey out of 39 surveys sent out. Small users were not surveyed. The demand reported is expected to grow at a yearly compounded rate of 21% from 2005 to 2009. Table 2 summarizes the reported findings.

Table 2. Projected growth rates in the use of organic sugar.

Year	Projected overall % increase	Projected total lbs. Purchased/sold Total per year	Projected total lbs. increased per year
2004	19%	66,176,023	---
2005	21%	79,990,528	13,814,505
2006	21%	96,900,788	16,910,251
2007	21%	116,941,722	20,040,944
2008	21%	141,124,693	24,182,971
2009	21%	171,252,357	30,127,664

The table above demonstrates a growth rate in the use of organic sugar, which is similar to the growth of the whole organic market in the U.S. The U.S. market growth is close to twice that of Europe, although Asia (Japan specifically) is developing rapidly, but from a very low base.

As the organic market matures, more products will be produced with the organic label, giving an alternative to the traditional products. One of the challenges faced by organic producers is the production of organic drinks. Liquid Organic Syrup (67.5 brix) and Liquid Organic Syrup Medium Invert (76.5 brix) will be the sweeteners used for carbonated and non-carbonated organic drinks. Organic medium invert syrup cannot be produced using the traditional mineral acid procedure (food grade hydrochloric acid). To maintain organic status, medium invert organic syrup has to be produced by using either an enzyme or an approved organic acid like citric acid for the partial inversion (~50% of the sucrose) of organic sugar syrup. Either method takes a longer time to produce the syrup, but is a viable option to maintain organic status. Due to the higher color of the organic sugar used to make this medium invert syrup the sensory characteristics will be different compared to traditional medium invert or liquid sucrose and must be factored into the formulation of the soft drink.

Organic syrup (67.5 Brix) can be made from organic sugar and both the organic medium invert and organic syrup will not be very close to the 45 color upper limit currently specified in the ISBT (International Society of Beverage Technologists) guidelines for granulated and liquid sucrose. Medium invert guidelines have not yet been developed by ISBT. The higher color (200 – 500 ICUMSA units) and the corresponding sensory characteristics due to the different flavor components present have to be taken into consideration in formulations of organic soft drinks.

In recent years the addition of organic powdered sugar (which contains 3% organic corn starch), organic brown sugar, organic Sucanat (a free flowing whole cane brown sugar), organic blackstrap molasses and organic turbinado (Raw Cane / Demerara Style products) and organic Sucanat with Honey add to the organic products currently available in the organic market place to both the industrial and retail consumers. This demonstrates the organic sugar industry is growing and rising to the challenges presented by the overall growth in the organic market.

There are also other organic sweeteners currently available, Organic Rice Syrup, Organic Fruit Juice Concentrates, Organic Agave Syrup (also called Agave Nectar), and Organic Corn Syrup. The Organic Rice Syrup and Organic Agave Syrup are already available in bulk to industrial users, and the Organic Corn Syrup may soon be available to the industrial market soon though high costs and market pricing are an issue

Recently large food chains in the U.S. have announced they are looking to capitalize on the current organic market growth potential by expanding the number of organic foods offered in their retail stores. This will fuel the continued growth of organic products many of which may use organic sugar as an ingredient. The main concern with this development is the very large chains could also put downward pressure on pricing to compete with traditional products and change some of the margins currently enjoyed by organic producers. This will be and interesting development to watch.

Conclusion

The organic consumer market is less than 3% of the whole food market in the U.S.; however, it has experienced a steady growth of approximately 20% annually for the last 10 years. There is no sign of this trend diminishing and the demand for organic sugar will grow along with the rest of the organic market. Organic sugar and organic sugar products are a very important part of the whole organic market, both as stand alone items and as ingredients in many organic foods.

Organic sugar is here to stay.

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The Sugar Scene in Europe

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Background

The Sugar Regime in Europe was established as part of the Common Agriculture Policy (CAP) in 1968, and has changed little since then. The intent of the regime was to "maintain employment and standards of living for European growers of sugar beet." It is a complex system of quotas and price control. The quota system consists of A quota, which covers sugar for consumption in the EU; B quota, which covers surplus sugar to ensure continuity of supply in low production years, and C quota, which is the surplus for sale on the world market. The intervention price defined the price of A and B sugars. The actual price of sugar in the EU was based on the intervention price, fixed by the Council each year, plus storage and transport levies, plus a regional pemium. The council also set the price that processors paid beet growers. All imports with the exception of ACP sugars were subject to tariff.

The European Sugar Regime

Reference Price ~$ 820

Preferential Imports
ACP raw sugar
2m mt

World Market Exports
1.3 m mt

Beet sugar production quota of
17.44 M mt

HFS quota
547000 mt

World Market Price $ 380
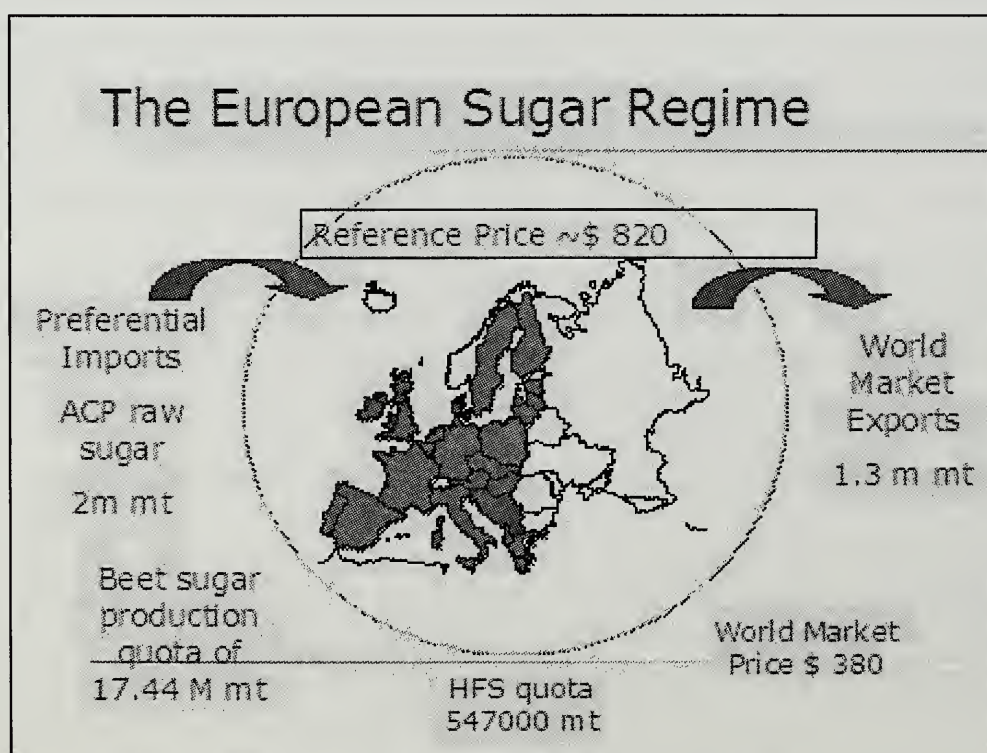
The regime set an intervention price of approx US$820 versus a World Market Price of approx US$380. It allowed preferential imports of 2 mm metric tonnes of raw sugar from the African Caribbean and Pacific countries, primarily the previous colonies of UK and France, who had a long established trade with the cane sugar refiners in Europe. Beet production quota for the EU countries was 17.44 mm metric tonnes.

The regime also allowed a High Fructose production quota of 547,000 metric tonnes.

An additional 1.3 mm metric tonnes of C sugar was exported onto the world market.

In the countries of the EU approximately 60 percent of the quota is in the hands of seven key suppliers (Table 1). Südzucker, by far the biggest supplier, includes Agrana in Austria, St Louis in France, and Tienan in Belgium.

Table 1. Quotas in Europe (% of European quota. Total: 17.44 mm tonnes)

1	Südzucker	Germany	23.45 %
2	Tereos	France	9.36 %
3	Nordzucker	Germany	8.83 %
4	British Sugar	Great Britain	7.61 %
5	Danisco	Denmark	6.57 %
6	Pfeifer & Langen	Germany	6.19 %
7	Ebro Puleva	Spain	4.49%

Drivers for Change

The time for change had arrived. The drivers for that change were the supply/demand equilibrium in the market and the impact of the WTO ruling on exports which ruled that C sugar exports onto the world market were illegal, as they were, in effect, subsidized by the high prices of A and B sugars within the community.

In addition, the EU had to adapt to its international import agreements, including the Balkan agreement and the "Everything but Arms" agreement for "Less Developed Countries." The new regime came into effect on July 1, 2006.

On July 19, 2005 the EU Commission presented a plan to radically reform the regime (Table 2). A new reference price would replace the intervention price. Intervention buying would be swept away and replaced with an aid scheme for private storage "when market price falls significantly below reference price" – this would apply on regional basis.

Table 2. EU Commission's Proposal

> Abolish intervention
> Cut prices by 39% in stages, commencing 2006
> Aid for beet growers
> Cut quotas by 2.8 million tonnes by 2008
> Increase HFS quota by 300,000 T
> Tradable quotas

The reference price would be immediately 20% below current intervention price and would be cut again in 2007 by a further 13% to €421.

Beet growers who left the industry would be compensated by a direct aid scheme. Production quotas would fall to eliminate export surplus. Outgoers scheme for inefficient producers – give up quota for €250/tonne or trade it.

Not part of the proposals, but expect the EU to cut import duties and increase duty free access for least developed countries.

The final proposals accepted by the council and commission is set out in Table 3.

Table 3. Reform measures.

National Quotas	Still in place until 2014/2015.
Voluntary exit scheme	Producers can opt out of sugar production with compensation. (2006 & 2007 = €730/mt , 2008 = €625/mt , 2009 = €520/mt)
Restructuring Fund	Levy added to producer reference price to finance 'opt out' compensation. (06/07= €126.4/T – 07/08 = €173.80/T – 08/09 = €113.30/T)
Additional quota	1 million mt additional quota for largest producers of C sugar at €730/mt. Remaining countries, 10,000 mt each. (Total 1.1M mt offered)
Institutional Price Levels	36% reduction (€632 to €404.40/mt) over 4 years (2006 – 2010). Consumers only see reductions after 2007/08.
Intervention	Maintained until 2010 but max. 600,000 mt at 80% of following year's reference price.
ACP Sugar	1.3 mm mt preferential sugar imports continues.
EBA Sugar	No Quota limitations on LDC sugars after 2009/2010
HFS	Additional 300,000 T over 3 years
C-Sugar Exports	From May 22nd 2006 the EU will stop issuing export licences for C sugar (WTO ruling)

National quotas will remain in place until 2014/2015. Producers will be able to opt out of sugar production with compensation on a reducing scale through to 2009.

A levy will be added to the producer reference price to finance the opt out compensation scheme. Additional quotas will be given to producers to compensate for loss of C sugar exports. Institutional price levels will reduce over four years to US$ 404.40 per metric tonne.

ACP sugar imports will be allowed to continue, and EBA (Everything But Arms) sugar imports will be allowed with no quota limitations after 2009/2010.

There will be additional quotas of 300,000 metric tonnes of HFS over the next three years, and as mentioned, C sugar exports will be stopped from May 22, 2006, in line with the WTO ruling.

LDCs - Everything But Arms

LDCs are a group of 49 countries judged on several criteria to be the poorest in the world. Mostly African countries – the EU offered comprehensive duty free access for " Everything But Arms". The import duty tariff will reduce over the next four years and import quotas will increase (Figure 1). Initially these imports will be limited to raw sugar imports to port refiners.
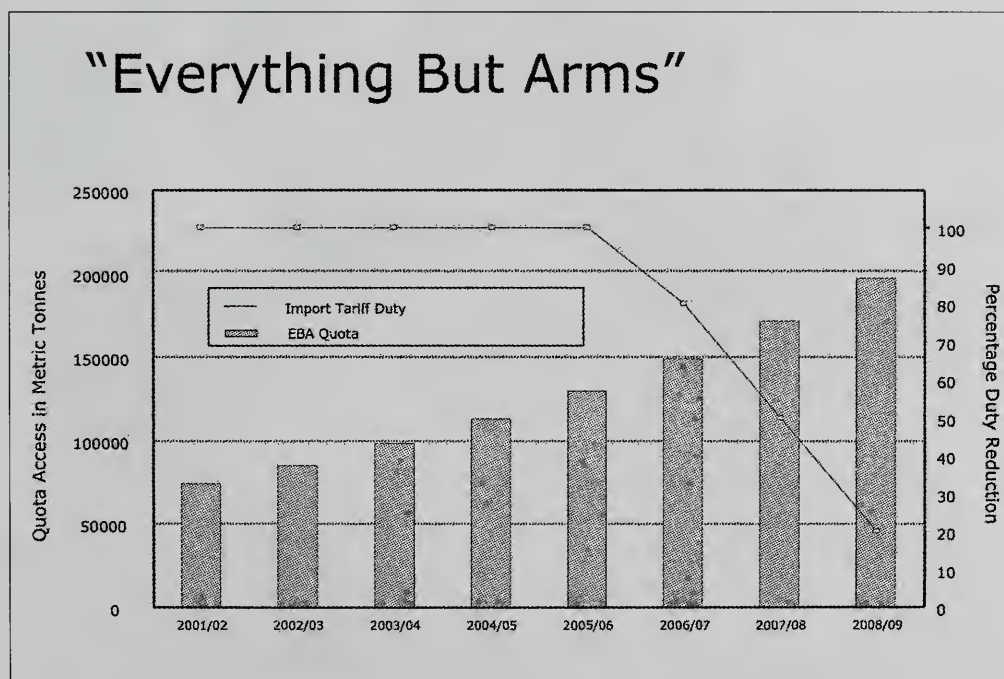
Figure 1.

The main producers are shown in Table 4. There are at least another dozen with some sugar production. Some of these are owned by international conglomerates. Between them they could easily supply over a million metric tonnes. Of course this will also impact EU industries and the EU's traditional cane sugar suppliers.

Table 4. LDCs export availability.

Country	Potential Production	Local/Regional Offtake	Net Export Availability
Bangladesh	200,000	800,000	0
Burkina Faso	40,000	60,000	0
Ethiopia	400,000	350,000	50,000
Malawi	300,000	130,000	170,000
Mozambique	400,000	120,000	280,000
Nepal	100,000	100,000	0
Sudan	1,200,000	800,000	400,000
Tanzania	300,000	300,000	0
Zambia	250,000	120,000	130,000
Total	**3,190,000**	**2,780,000**	**1,030,000**

EU Sugar Market

Some EU producers are high cost and could well be uneconomic if the price is cut by 15-20%. A further group would become uneconomic if cuts exceed 20% (Figure 2).
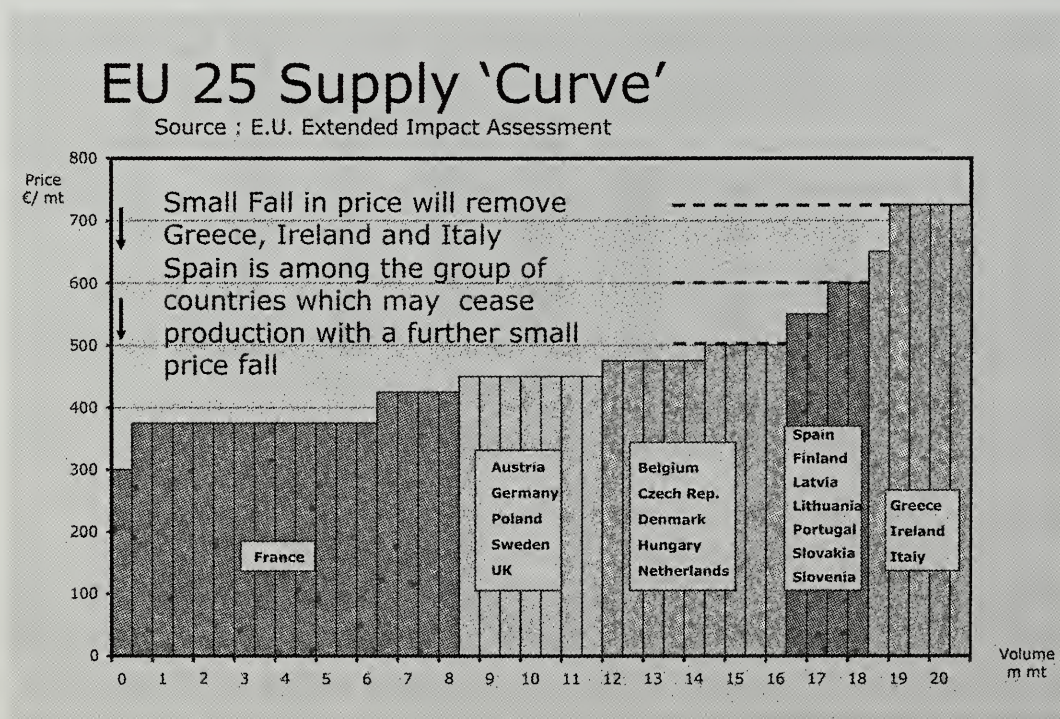
Figure 2.

As the market price falls to the new proposed reference price level of €404, the only viable beet industries would be in a swathe across central Europe running from Norfolk in the UK to Poland. Production could rapidly fall by several million tonnes.

Some traditional cane sugar suppliers would also disappear, while other low cost producers would expand sales, as would some LDCs.

In 2006/7 the sugar market across Europe will look like Figure 3. Some Mediterranean countries, Ireland, Finland and perhaps the Baltics, will be in deficit, whilst countries across Central Europe will be in surplus. However, by 2010/11, the market is expected to have changed with more Northern European countries in deficit (Figure 4).
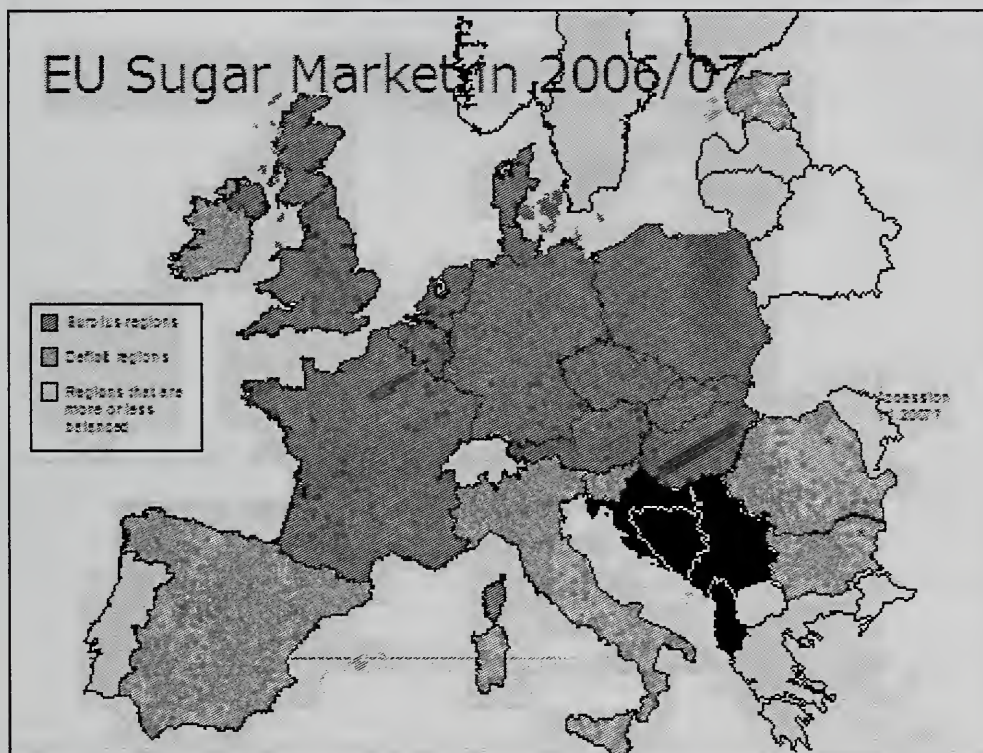
Figure 3.
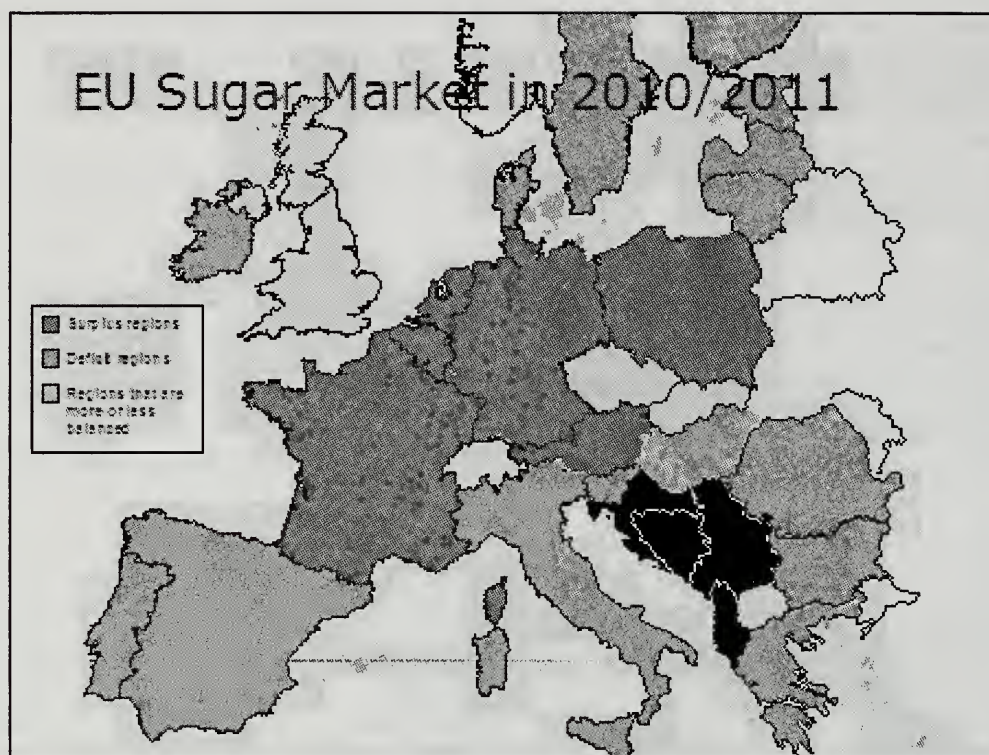
Figure 4.

Impact on Cost of Sugar Production

What will the impact of the changes be on sugar production costs?   The reference price reduction will be 90€ per tonne higher than beet cost reduction, which will impact profitability. Energy and freight costs will continue to rise and, in addition, the restructuring fee must be paid on the full sugar quota (+20-30 €/t for each ton sold), not on sugar sold.

Potential Impact on the Sugar Industry

It is likely that only the most competitive countries will survive, and some sugar producers will make use of the restructuring funds and reduce or even stop producing. In 2006/7, there are 50 players in sugar processing in Europe. By 2010/11it is expected that this will be reduced to 10 players, and the top 5 players will control 75% of the market.

2006/2007 Summary

To summarize, 2006/7 EU crop expectations based on plantings will be 15.5-16.5 metric tonnes. Italian production is already down 70% in 2006/7 versus 2005/6, with 13 out of 19 factories closing. Ireland and Slovenia will cease beet sugar production altogether in 2006. Sweden will surrender 40,000 metric tonnes of quota, and it is expected that further quota across the EU will be given up in 2006/7 with further announcements expected. There has already been some consolidation, with Nordzucker of Germany, Cristal Union of France and E.D.&F. Man of the U.K., recently creating a joint sales company – Eurosugar.   More HFS will be available

Impact on Customers

Finally, what will be the impact on customers?

Customers will not see institutional price reductions until 2008/09. There is uncertainty today regarding total volume of quota which might be surrendered.

The 2007 and 2008 internal market prices will depend on the internal supply situation which will be impacted by the level of intervention stocks, carry-over stocks from the previous year, the number of players left in the market, and finally the quota surrendered and quota cuts. The changes will certainly bring about a general equilibrium in the market at the expense of regional instability. Freight costs will play and increasingly important role on the customer in the EU market

Quality Improvement of Raw Sugar: Working with Your Supplier

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Abstract

Raw Sugar quality is a governing factor controlling the processes in any sugar refinery. For a given raw sugar, the levels of color, turbidity, ash, starch, dextran, sulfite and insoluble matter are major quality parameters affecting the refining process, technically and economically, positively at their low levels and negatively at high levels. In spite of many new developments achieved in sugar refining technologies, some refiners are interested in having superior quality raw sugar to cope with the developing programs adapted in their refineries. Removal of raw sugar impurities is easier in the sugar mill than in the sugar refinery. In the cane sugar mill, sugar cane is the raw material for producing raw sugar. Following good manufacturing practices throughout the mill is strongly recommended. Implementing proper monitoring and management systems for activities concerning sugar cane with respect to maturity, harvesting, transporting fresh healthy cane, washing, crushing and finally processing high purity raw juice with selected way of clarification, concentration and sugar crystallization, are the best contributors to high quality raw sugar. For the raw sugar producer, a good understanding of refiner needs and working to fulfill them economically by following a suitable program for quality improvement mutually agreed by both parties gives both of them a lot of benefits .

Al Khaleej Sugar Company, Dubai – UAE, as a refiner made a successful arrangement with Copersucar, Brazil, which is their major supplier of raw sugar. Since 2000 they have worked together and hit the set of targets for improving the quality of VHP (Very High Polarization) raw sugar appreciably and produce VVHP (Very, Very High Polarization) raw sugar economically. VVHP raw sugar has had many valuable impacts on Al Khaleej Refinery. This paper describes briefly the program of improving raw sugar quality applied successfully in sugar mills belonging to Copersucar.

Introduction

The major raw material for a sugar refinery is "Raw Sugar". The selection of a technological path and operational parameters of highly efficient and economical refining processes are highly dependent upon the quality of raw sugar to be processed. On the other hand, the quality of raw sugar produced in the sugar mill depends on the quality of processed sugar cane and process variables of mill operation.

Sugar cane quality is related to many factors such as cane variety, soil, climatic conditions, irrigation, fertilizers, management of field operations, maturity tests, harvesting transport, etc.

Al Khaleej Sugar (AKS) / Dubai – UAE, was commissioned as a sugar refinery in 1995 to process 2400 tons / day of conventional type raw sugar. Improving refinery processes was and remains the main issue considered the top priority by the innovative & dynamic company management. The Idea of processing High Quality Raw Sugar was put forward as a major target to improve refinery processes to avoid the many drawbacks of refining lower quality raw sugar. To implement this, VHP (Very High Polarization) raw sugar was imported in 1997 and mixed with low purity raws to feed the refinery. Valuable impacts were gained, such as, eliminating the affination process and boiling A- Massecuite in batches instead of continuous boiling, which gave additional capacity to refined sugar boiling, i.e. increasing the production of refined sugar.

As improvement is a continuous process, AKS had the intention to have raw sugar with better quality than VHP that needed to produce raw sugar covering higher quality parameters, in turn this trend needed to have a suitable arrangement with a raw sugar producer. Therefore, AKS selected Copersucar / Brazil as its major raw sugar supplier, to achieve the required goals, so that a fruitful collaboration program was decided and implemented successfully between the two parties through a signed Memorandum of Understanding to produce raw sugar with quality higher than VHP raw. Copersucar named its sugar "VVHP" (Very, Very High Polarization) raw sugar. In 2000, production of VVHP raw sugar was started by Copersucar and refined by AKS with appreciable impact.

Currently another Program between both parties is in progress to improve VVHP quality.

This paper will discuss briefly the practices followed in Copersucar's sugar mills to produce VVHP raw sugar.

Why AKS Was Interested to Have Superior Quality Raw Sugar for Refining Other than Low Purity Raws and VHP Raw Sugar

AKS Refinery uses carbonation as the mode of purification. Affination process as a way to improve the quality of raw sugar before melting was followed during the period July 1995 - August 1997. Affination requires a lot of equipment and demands high power and regular maintenance. Affination wash had high color and high content of impurities. To recover maximum sugar out of it economically, many boiling and recycling steps were required,

consuming appreciable amounts of electrical power and steam in addition to increasing the recycling of impurities in the refinery.

Affined raw sugar, though, had better quality than the raw itself (concerning color, insoluble matter, starch, dextran, turbidity-contributing components, and other non sugars), but still had many other impurities that created a heavy load on the carbonation process, consuming higher quantities of quicklime and washed carbon dioxide, in addition to less throughput in filtration, giving short working cycles to granular activated carbon columns, and consequently the number of cycles for carbon regeneration process increased with high carbon losses. Color of decolorized liquor was only suitable to boil two refined sugar massecuites, R1 & R2. R3 & R4 sugars were used in magma preparation. In the recovery house A, B and C boilings were being done. It was noticed that the refinery was building up non sugars and color in materials under processing.

It is well known that starch and dextran have a bad effect on refining processes. Affined sugar had measurable contents of these polysaccharides. α – Amylase enzyme was used to reduce the amount of starch before carbonation. Since dextranase enzyme to reduce dextran is not suitable for the refining process, it was not used (it works more efficiently under the manufacturing conditions of raw sugar mills).

Thus, no solution was available in the AKS refinery for this problem. Therefore, AKS was convinced that it had to process raw sugar with high polarization, low color (provided that sulfur dioxide is not used in the process to reduce the color), and minimum levels of starch, dextran and insoluble matter. Consequently, AKS started to process VHP raw sugar, which had better quality than low polarization raw sugar, to avoid the drawbacks of processing low quality raw sugars.

VHP refining produced many valuable impacts on the overall processes, such that full confidence was created in AKS which can be stated as **"Reducing the impurities of raw sugar is a governing factor in improving performance of the sugar refinery and can be achieved easily & economically in the sugar mill."**

Accordingly, to have more good impacts on the refining processes, the opinion of getting raw sugar with quality better than VHP raw sugar was initiated, and priority was given to find suppliers interested in producing such raw sugar.

AKS arranged successfully in this regard with Copersucar, a well-known Brazilian supplier of raw sugar, which agreed with the idea to proceed forward to provide raw sugar with quality better than VHP raw to meet AKS needs.

Types of Raw Sugar Processed in Al Khaleej Sugar (AKS):
1- Low Polarization (LP) Raw Sugar
2- Very High Polarization (VHP) Raw Sugar
3- Very, Very High Polarization (VVHP) Raw Sugar

The average quality parameters of these types of sugars is shown in Table 1.

Table #1. Average quality parameters of sugars processed by AKS.

Raw Sugar Type	LP	VHP	VVHP
% Polarization	98.36	99.40	99.63
% Moisture	0.30	0.06	0.04
% Ash	0.26	0.12	0.07
% R.S.	0.47	0.13	0.07
% R.S. / Ash	1.80	1.08	1
Color (ICUMSA)	3800	800	427
Insoluble Matters[on8µm]	N.A.	250	150
Starch (mg / kg)	N.A.	237	125
Dextran (mg / kg)	N.A.	< 50	< 50
Sulfite (mg / kg)	N.A.	N.A.	Nil

N.A. = Information not available.

Mixing different types of raw sugars is also being followed in AKS refinery to maintain the refining process without affination.

Quality of sugar materials during refining three types of raw sugar following different combinations in the melter

LP   With affination + A – Melt
VHP   Without affination + A – Melt
VVHP   Without affination + R6 – Melt

The quality of sugar materials from refining the three types of raw sugar are shown in Table 2.

Copersucar / AKS Program to Have Raw Sugar with Better Quality Than VHP Raw Sugar

The program was managed through a signed memorandum of understanding between the two parties. A work team was named and headed by a Copersucar representative. The required specifications for raw sugar were fixed by AKS and agreed by Copersucar, and a mutual decision was taken to work directly with the sugar mills belonging to Copersucar in order to produce raw sugar with the required quality. Copersucar called it Very, Very High Polarization (VVHP) raw sugar.

Table 2. Quality of sugar materials from refining the three types of raw sugar

Description	LP Raw sugar				VHP Raw sugar				VVHP Raw sugar			
	Pty	Col.	Ash%Bx	R.S.%Bx	Pty	Col	Ash%Bx	R.S.%Bx	Pty	Col.	Ash%Bx	R.S.%Bx
Raw sugar melt	99.3	2128	0.3	0.28	99.4	1500	0.14	0.27	99.5	1000	0.06	0.06
A - Melt	99.2	4090	0.32	0.31	99.26	2726	0.13	0.25			x	x
R6 - Melt	x	x	x	x	x	x	x	x	99.5	1520	x	x
Carbonated Filtered Liquor		844	0.28	0.28	x	473	0.24	0/1	x	203	0.21	0.06
Fine liquor		175	0.28	0.29	x	154	0.24	0.1	x	90	0.11	0.06
R1 - Mass.	99.3	x	x	x	99.4	329	x	x	99.5	127	x	x
R2-Mass.	98	x	x	x	x	x	x	x	99.12	110	x	x
R3-Mass.		x	x	x	x	x	x	x	97.74	1182	x	x
R4-Mass.		x	x	x	x	x	x	x	95.21	3021	x	x
R5- Mass.		x	x	x	x	x	x	x	90.68	6840	x	x
R6-Mass		x	x	x	x	x	x	x	82.98	13359	x	x
A - Mass.	87.2	x	x	x	x	x	x	x	x	x	x	x
B - Mass.	72	x	x	x	x	x	x	x	x	x	x	x
C- Mass.	60.5	x	x	x	x	x	x	x	x	x	x	x
Final Molasses	52.1	x	x	x	54	x	11.15	18.27	54	x	11.35	19.48
R6 - Run off or Sugar syrup	x	x	x	x	x	x	x	x	x	102925	x	x

The program covered main issues starting with sugar cane up to sugar handling and can be represented by the following points:

1- Time of sugar cane cutting:
To have best juice purity suitable to produce high quality raw sugar, sugar cane must be mature, so that the time for sugar cane cutting is planned to start at the peak of cane maturity, generally not at the beginning of the crop season in which sugars other than VVHP or alcohol can be produced by the mills.

2. Cane transport:
Quickest practice to transport cane for milling was recommended, to prevent cane deterioration and dextran formation.

3. Dextran control:
During rainy periods, dextran content of first expressed juice was monitored and controlled by adding calculated amounts of dextranase.

4. Cleanliness of the sugar cane:
Cane leaves, tops and field trash were minimized as per the available possibility before cane crushing to reduce non-sugar content (such as starch, coloring matter, and turbidity contributing components) of the juice.

5. Cane washing:
Proper cane washing, using clean water before cane preparation for crushing was adopted during rainy periods to reduce the load of insoluble matter as well as turbidity in the juice.

6. Juice to be processed:
Primary juice was processed with priority to produce VVHP raw sugar. Minimum mixing of other juices or recycling is recommended with any recycled product (such as sweet water from vacuum filters).

7. Mill Sanitation:
Restricted program for mill sanitation with approved chemicals, hot water and steam is carried out, to control dextran formation during crushing.

8. Primary juice clarification:
Proper ways of treatment in this stage were implemented.

9. Clarified juice screening:
Different methods of screening clarified juice were followed in different mills, mainly to reduce as much as possible bagacillo particles.

10. Controlling starch content:
Starch content of concentrated clarified juice (syrup), was controlled by treatment with α-amylase enzyme. A well-known brand of enzyme was selected; a method for most efficient usage was found after intensive trials.

11. Sugar boiling:

High purity syrups were boiled with low recycling and mixing of low purity materials during boiling stage; magma preparation also was made from high purity materials; mean aperture of the crystals was controlled as large as possible, leading to a need of more cutting in A & B strikes.

13. Sugar washing in centrifugals:

Well selected operational parameters for separation in centrifugals like spinning, washing, and wash water temperature, were implemented to remove maximum amounts of mother liquor and insoluble materials from the outside surfaces of the crystals and turbidity contributing components.

14. Sugar drying:

Proper sugar drying and cooling were established, to have good quality for storing, as well as to prevent sugar caking problems.

15. Sugar conveying:

Minimize the number of conveying processes to convey wet sugar from centrifugals and dry sugar from dryer to the stores to prevent formation of sugar dust.

16. Sugar handling:

VVHP Raw Sugar was handled carefully from the mill to the terminal to prevent crystal breakage and sugar dust formation, and GMP applied to avoid any possibility of external contamination of the sugar produced.

Some contributions of different types of raw sugar on refining processes:

1- Reducing the number of recovery house massecuites % refined sugar produced:

Raw sugar	LP	VHP	VVHP
A-Massecuite	33%	7%	5%
B-Massecuite	8%	6%	2%
C-Massecuite	6%	4%	0%

2- Increasing the cycle time of granular activated carbon columns:

Raw Sugar	Cycle time (hrs)
LP	400 Max.
VHP	600 - 800
VVHP	> 1500

3- Reducing energy & water consumption / ton refined sugar:

Reference year	Raw sugar	Fuel Kg/ton	Water Lts./ ton	Power KW hr / ton	Steam Kg / ton
1997	VHP	77	392	121.54	938
2005	VVHP	38	164	50.51	509

Conclusion

Working of the Sugar Refiner with the Raw Sugar Supplier to achieve challenging specification and improving the quality of raw sugar is a mutually profitable project for both parties.

Alicyclobacillus (ACB) Species: A Growing Concern for Manufacturers of Shelf-Stable, High-Acid, Non-Carbonated Beverages

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Abstract

Alicyclobacillus (ACB) species are thermoacidophilic sporeforming bacteria of increasing concern to the beverage industry because of their ability to survive conventional pasteurization treatments and to germinate and grow in hot-filled and aseptically processed high-acid beverages, without producing overt signs of spoilage. ACBs are widely distributed in nature, and can be found in a variety of raw agricultural commodities, as well as in the food and beverage ingredients derived from them. At particular risk to shelf-stable, high-acid, non-carbonated beverages are ingredients whose starting materials are harvested in direct or in close contact with soil, such as fruit juices, teas, herbs, and sweeteners. This presentation addresses the sources and significance of ACBs in sweeteners and provides an overview of the control strategies needed to minimize finished beverage ACB spoilage risk.

Outline
Alicyclobacillus (ACB) spp. characteristics
Significance of ACB to the beverage industry
Sources and risk factors in sweetener production
Risks from other ingredients, environment and processing
Control strategies
Summary

Alicyclobacillus spp. characteristics
Non-pathogenic sporeformer, soil-borne
ω-alicyclic fatty acids in cell membrane
20 recognized species, four are of concern to industry
Thermotolerant/thermophilic (Growth range: 25°- 65° C; optimum: 45°- 50°C
Acidophilic: pH range: 2.0 - 6.0; optimum: 3.5 - 4.0
Aerobic/microaerophilic
No growth in concentrates >30°Brix

Beverages susceptible to Alicyclobacillus spoilage
Shelf-stable, high acid, non-carbonated (hot-filled and aseptic)
Juices and juice-containing
Isotonic/sports drinks
Acidified teas
Formulations containing precursors of taint compounds

Why is Alicyclobacillus a concern in hot-filled and aseptic beverages?
Spores are ubiquitous and resistant to heat, acid, concentration and drying.
Pasteurization can trigger germination.
Growth occurs over wide pH and temperature ranges.
Lack of additional "hurdles".
Oxygen ingress into package.
Overt signs of spoilage are absent, and spoilage is evident only at time of use.

Alicyclobacillus spp. spoilage manifestations
A. acidoterrestris is usually implicated. However, its presence doesn't always mean spoilage; and
    multiple species could be present.
Spoilage is temperature, time and nutrient dependent.
No gas/pH change, little or no sediment is formed.
The main problem is a medicinal, chemical, disinfectant taint/odor caused by compounds with
    extremely low odor thresholds: Guaiacol = ppb (μg/L) and halophenols = ppt (ng/L).

Table 1 lists sources and risk factors in beet and cane production. Arrows indicate the direction of
the risk; an up arrow indicates increased risk; a down arrow indicates decreased risk; a two-headed
arrow indicates that risk can go either way depending on conditions. Table 2 lists sources and risk
factors for high fructose corn syrup (HFCS) production.

Table 1. ACB sources and risk factors in beet and cane sugar production

- Harvesting (soil/sand ↑)
- Preparation (wash/rinse ↑, press/shred ↑)
- Extraction/diffusion (recirculation water ↑, acid ↑)
- Purification (lime ↓, carbonation ↓, clarification ↕)
- Softening/ion exchange (temperature ↕)
- Evaporation (rate/solids ↕)
- Crystallization (feed/remelt syrup ↕, magma temperature ↑, cooling temperature ↑)

Table 2. ACB sources and risk factors in HFCS production

- Harvesting (soil/dust) ↑
- Slurrying (temperature ↑ pH ↑)
- Liquefaction/dextrination (temperature ↓ pH ↓)
- Decolorization (temperature ↕, acid ↑)
- Cooling (temperature ↑ acid ↑)
- Heat exchange (temperature ↑ acid ↑)
- Isomerization (alkali ↓)
- Evaporation (pH ↓)
- Blending/transport (cross-/re-contamination ↑)

ACB risks from other ingredients, environment, and processing

Ingredients
    Liquid (water, juices, sweeteners)
    Dry (flavors, colors, herbs/teas, salts, functional agents)
Environment
    Soil, air, water, equipment
ACB-selective processing steps
    Concentration/dehydration, acidification, pasteurization, hot-holding, slow-cooling

A total systems approach is necessary for controlling Alicyclobacillus spp.

Patterned after HACCP (Hazard Analysis of Critical Control Points).
Identify all system inputs/outputs
    External/internal
    Engage all disciplines/levels
    Process flow charts
Assess/assign risks
Best practices lists

Alicyclobacillus control strategies for concentrate and bottling plants

Reduce incoming spore loads
    Manage supply; "zero tolerance" impractical
        Vendors, specifications
    Ingredient pre-treatment/replacement
        Consider impact on sensory, quality, functionality
    Equipment design, cleaning, sanitation
        Prevention, sustainability
        Sanitizer efficacy studies (ISBT ACB Control Subcommittee)
    Environmental monitoring: Eliminate niches and re-contamination.

Product reformulation: Eliminate guaiacol precursors (vanillin, vanillic acid, ferulic acid); add
    preservatives (e.g., nisin)

Process re-evaluation and interventions:
> More robust thermal process
> Non-thermal alternatives (e.g., filtration)
> Hybrid processes
> Product cooling rate

Package re-evaluation
> Materials/barriers
> Oxygen scavengers

Storage and distribution temperatures
Business model/product turn rate
Global collaboration via ISBT ACB Control Subcommittee (International Society of Beverage Technologists).

Alicyclobacillus spp. specification considerations
Methodology issues (ISBT Methodology Sub-committee)
> Global acceptance
> Rapid, reliable, reproducible
> Sample size
> Quantitative vs. qualitative
> Confirmation
Sampling/testing frequency (unfeasible by lot)
Acceptance criterion (genus? sp.? guaiacol?)
ISBT ACB culture collection (in progress)

Summary

Many Alicyclobacillus spp. are found in the environment, and only some cause spoilage – target guaiacol-producing *A. acidoterrestris* strains.

Availability of a universally-accepted test method is critical for risk assessment, specifications development, and process control (an ISBT initiative is in progress).

Many commonalities exist between supplier and customer production practices – use to assess industry risks and develop global best practices (an ISBT initiative is in progress)

Lot-by-lot testing for ACB is unfeasible and "zero tolerance" standards are impractical.

A total systems approach is the only viable control strategy.

The Expanding World of Nutritive and Non-Nutritive Sweeteners

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Abstract

The market for sweeteners is estimated to grow at about 8.3 percent per year up to 2008. [1] This growth is fueled in large part by rising health concerns about diet and obesity. The sweetener industry is composed of a complex and sometimes confusing array of nutritive and non-nutritive sweeteners, which includes lower intensity sweeteners and very high intensity sweeteners. Besides the traditional carbohydrate sweeteners – sucrose, glucose, fructose, honey and molasses – there are sugar alcohols, new sugars, such as tagatose and isomaltulose, and at least one new very high intensity sweetener, Neotame, 8000 times sweeter than sucrose. To complicate matters more, some of the sweeteners go by several names or have brand names. Some of the newer sweeteners also have additional functions as pre-biotics or as food fiber. The latest trend in sweeteners is the production of blends, combinations of nutritive and non-nutritive sweetener ingredients designed to produce the desired sweet taste with fewer calories or slower digestibility and other functionalities. This paper provides an overview of the different types of approved sweeteners on the market, their relative sweetness and functionality, and will discuss some of the new commercial blends.

Mention of trade names and commercial products in this article is solely for the purpose of providing information and does not imply recommendation or endorsement by Sugar Processing Research Institute, Inc.

Introduction

Consumers remain generally positive toward sugar, and either have a fairly negative view of (artificial) sweeteners, or are concerned that they don't understand enough about them to assess the long-term health implications, particularly for their children, of using them. [2] Nevertheless, there is a growing market for non-sucrose sweeteners. Obesity trends have increased product development efforts to replace caloric bulk sweeteners with alternative sweetening systems without changing the full sensory experience of sweetness and mouthfeel.

Types of Sweeteners

Sweeteners can be categorized in a number of ways: (1) by structure, as carbohydrates or non-carbohydrates; (3) by degree of sweetness, e.g., high-intensity or low intensity; or (4) by calorie count, e.g., caloric, reduced calorie, or non-caloric.

Sucrose has always been considered the "gold standard" of sweeteners and all sweetener ingredients are compared to sucrose in terms of its sweetness, bulking properties and other functional qualities. The sweetness of sucrose is set as 1.00. Sucrose has five structural isomers, four of which have been commercialized to one degree or another. Table 1 lists the sucrose isomers.

Table 1. The structural isomers of sucrose.

Isomer	Sweetness	Structure	Status
Sucrose	1.00	(α-1,2)	Commercialized
Leucrose	0.37	(α-1,5)	Commercialized
Maltulose	0.42	(α-1,4)	Commercialized
Isomaltulose	0.3-0.4	(α-1,6)	Commercialized
Trehalulose	0.6-0.7	(α-1,1)	Commercialized
Turanose	"very sweet taste"*	(α-1,3)	Not commercialized

* Merck Index, Twelfth Edition, 1997

Polyols / Sugar Alcohols

Polyols are a category of bulk nutritive sweeteners known as sugar alcohols. Sweetness levels vary from about 0.3 to 1.0, depending on the polyol. Sweetness levels also may vary somewhat depending on their concentration and usage, which explains why a range of sweetness is sometimes listed. Polyols are excluded from the term "sugar" on ingredient labels, so foods containing sugar alcohols can claim to be "sugar free." They have fewer calories than sucrose and are slowly absorbed, which means they have little effect on insulin metabolism and can be used by diabetics. Labeling legislation in the European Union requires that all polyols show a caloric value of 2.4 kcal/g, but other labeling systems (such as U.S., Australia and Japan) permit specific caloric values for individual polyols. [3] When put into solution, they have negative heats of solution, resulting in a cooling effect in the mouth, which may or may not be desirable, depending on the product. Sugar alcohols may have a laxative effect if eaten in excess, and recommended daily intakes are in place for some. They are generally non-cariogenic (do not cause tooth cavities). They are highly soluble and non-hygroscopic. The sugar alcohols are non-reducing, temperature stable and more resistant to browning reactions than sucrose, which may or may not be an advantage, depending of the usage. The approved sugar alcohols are: erythritol, isomaltitol (isomalt/Palatinit™), lactitol, maltitol and maltitol syrups, mannitol, sorbitol, xylitol and hydrogenated starch hydrolyzates (HSH).

Erythritol

Erythritol is a polyol bulk sweetener which can be blended with low-calorie sweeteners and/or other polyols. It is a reduced-calorie sweetener with 0.2 kcal/g and is 70% as sweet as sucrose. It is said to have a clean, sweet taste with no aftertaste and is safe for diabetics. Erythritol has the lowest heat of solution of the polyols (highest cooling effect on dissolution), which is a consideration in product formulation. Commercial erythritol is a product of Cargill, with the product name of Eridex. Because erythritol is rapidly absorbed in the small intestine and rapidly eliminated by the body within 24 hours, laxative side effects sometimes associated with excessive polyol consumption are less likely when consuming erythritol containing foods (Calorie Control Council). [4] It is produced by the fermentation of glucose by yeast-like fungi, such as *Monieliella pollinis* and Aureobasidium sp. It has had GRAS (Generally Recognized as Safe) status in the United States since 1997.

Hydrogenated Starch Hydrolyzate (HSH) Syrups [5]

HSH syrups are a mixture of various chain length sugar alcohols. They are manufactured by the partial hydrolysis of corn, wheat or potato starch with acid or enzymes to produce specialized sugar syrups. The mixture of sugars in the produced syrup is hydrogenated to form their corresponding sugar alcohols. The composition of HSH syrups can vary widely depending on the functional properties desired, and some manufactures have large lines of HSH syrup products. Dried or powdered HSH compositions have also recently become available. HSH compositions are described based on the amount of different chain length saccharides in them. By varying the conditions and extent of hydrolysis, the relative occurrence of various mono-, di-, oligo- and polymeric hydrogenated saccharides in the resulting product can be tailored for various levels of viscosity, sweetness, humectancy and other properties. "Hydrogenated starch hydrolyzate" is more commonly used to describe the broad group of polyols that contain substantial quantities of hydrogenated oligo- and polysaccharides in addition to any monomeric or dimeric polyols (sorbitol/mannitol or maltitol, respectively). HSH syrups do not crystallize and help to prevent crystallization in products. Calorie content and digestibility are dependent on the composition. HSH can also be used to replace corn syrup in many applications. In the United States, HSH are provided by SPI Polyols, Roquette America, Inc. and Archer Daniels Midland Company (ADM).

Isomalt / Isomaltitol / Palatinit™

Isomalt is a nonhygroscopic disaccharide polyol bulk sweetener which can be used as a one-to-one replacement for sucrose. Products made with isomalt are said to have the same texture and appearance as those made with sucrose. Isomalt is a mixture of two disaccharide alcohols: gluco-mannitol and gluco-sorbitol. It is currently used in more than 70 countries and has been GRAS in the United States since 1990. Isomalt is produced from sucrose, which is first enzymatically isomerized to isomaltulose and then hydrogenated to make the polyol. Isomalt does not have the cooling effect of many other polyols. Like many sweeteners, the sweetness of isomalt depends on its concentration, temperature and the form of the product in which it is used. It is 45-65% as sweet as sucrose and contains 2.0 cal/g, is slowly digested, and has a low glycemic response. Isomalt behaves like a dietary fiber in that it is broken down by the gut bacteria and promotes the growth of beneficial bifidobacteria in the large intestine. It is marketed in the U.S. by Palatinit of America, Inc., under the trade name Palatinit™.

Lactitol

Synonyms: **Lactit, Lactositol, Lactobiosit**.　Lactitol is 35-40% as sweet as sucrose and has 2.0 kcal/g　Lactitol is derived from the hydrogenation of the glucose portion of lactose (a galactose-glucose disaccharide) obtained from milk.　It has the least amount of cooling sensation of the polyols. [3] It has been GRAS in the United States since 1993.　Lactitol is said to have a clean sweet, sugar-like taste with no aftertaste.　It is fermented in the colon and consequently has beneficial effects on the colonic microflora and functions as a prebiotic.　Its mild sweetness may be desirable in some products and also makes it a good bulk sweetener to blend with high intensity sweeteners. Danisco Sweeteners markets lactitol in both anhydrous and monohydrate forms and Purac markets several forms of lactitol under the trade name Lacty®.

Maltitol

Of the polyols, maltitol has the closest characteristics to sucrose in most applications. [6] Maltitol is produced by the catalytic hydrogenation of high maltose corn syrup. Maltitol is available in crystalline form or syrups ranging from 50 to 89% in maltitol purity. It is claimed that maltitol has no aftertaste like some of the other polyols do. Like other polyols, maltitol is slowly absorbed by the system.　Maltitol is about 90% as sweet as sugar and provides 3.0 calories per gram.

Mannitol

Mannitol is produced commercially by catalytic hydrogenation of fructose-containing syrups or of invert syrup; sorbitol is a co-product.　A new fermentation process has recently been developed. It has 1.6 kcal/g and is 50-72% as sweet as sugar.　Because of its slow absorption, excessive consumption may have a laxative effect, and products containing mannitol must include a laxative warning on the label if the mannitol content in a serving exceeds 20g.

Sorbitol

Sorbitol is about 60 percent as sweet as sucrose and has 2.6 kcal/g and a moderate cooling sensation. Sorbitol has been used in processed foods for at least half a century and occurs naturally at fairly high levels in a variety of fruits and berries.　Sorbitol may be synergistic with other sweeteners.　It is produced by the hydrogenation of glucose.　It finds uses as a humectant and texturizing agent in foods.　Sorbitol is very stable and can withstand high temperatures and does not participate in Maillard (browning) reactions. The U.S. Food and Drug Administration's regulation for sorbitol requires the following label statement for foods whose reasonably foreseeable consumption may result in the daily ingestion of 50 grams of sorbitol: "Excess consumption may have a laxative effect."

Xylitol

Xylitol has been used as a sweetening agent in human food since the 1960s.　Its greatest claim to fame has been as a noncariogenic sweetener (reduces development of dental cavities) as well as reduced plaque growth.　It sweetness is 95-100% that of sucrose, making it the sweetest of the polyols.　It has a strong cooling effect, although not quite as much as erythritol. [3] Calorie content is variously reported as 2.4 and 3.0 kcal/g.　It is produced from hydrogenated xylose.　(The polysaccharide, xylan, is obtained from birch trees and other plant sources, and is hydrolyzed to xylose for hydrogenation.) Xylitol is most often used in chewing gums and candies where a cooling effect is desirable.　It is said to mask off-tastes, such as bitterness and astringency, and to adjust the temporal profiles of sweetness perception in blends.　Xylitol is produced by Danisco.

The Newer, Novel Sweeteners

In recent years, a number of new, novel sweeteners have come on the scene. These include fructo-oligosaccharides (FOS), isomaltulose, lactulose, leucrose, tagatose and trehalose.

Fructo-oligosaccharides (FOS)

Also known as **inulin syrup, oligofructose, neosugar,** and **short chain fructose polymers**. Inulin is a heterogeneous blend of fructose polymers found widely distributed in nature as plant storage carbohydrates. Oligofructose is a group of polymers with a degree of polymerization (DP) <10. Inulin and oligofructose are not digested in the upper gastrointestinal tract, so they have reduced caloric value. Fructo-oligosaccharides (FOS) typically refer to short-chain oligosaccharides containing from 2 to 4 fructose molecules attached to a glucose molecule. FOS are produced on a commercial scale from sucrose using a fungal fructosyltransferase enzyme. FOS containing 2 fructose residues is abbreviated GF2 (G is for glucose, F, for fructose). Those with 3 fructoses are abbreviated GF3, and those with 4 fructoses, GF4. Similar molecules are obtained by partial enzymatic hydrolysis of inulin. Sensus America produces a number of sweetener products extracted from chicory root, including a liquid product called Frutalose L85, consisting of short-chain fructose polymers with 50% the sweetness of sucrose.

Isomaltulose (Palatinose™)

Isomaltulose/Palatinose™ is one of the five isomers of sucrose. It received GRAS status in March, 2006. In July 2005 the product received novel food status, the European equivalent of GRAS, and in Japan it has been used as a food ingredient since 1985. It is 30-40% as sweet as sucrose and contains 2 kcal/g, and has a low glycemic index. The manufacturers claim that it has a smooth sweetness profile similar to that of sugar, but with more scope for flavor development. It is produced by Palatinit, a subsidiary of Südzucker AG.

Lactulose

Lactulose is a disaccharide composed of galactose and fructose and formed by the isomerization of lactose when milk is heated. Its relative sweetness is 60% that of sucrose and it contains only 0.2 kcal/g. Currently, lactulose is used mostly for medicinal purposes, to treat liver and renal disease, and as a laxative. It is not broken down in the small intestine, but is fermented in the large intestine, and thus falls into the category of prebiotics. In the United States, lactulose is a prescription drug, and its use requires medical supervision. Use as a dietary supplement is considered experimental. Lactulose is available in some functional foods and nutritional supplements in Japan at a rate of about 2 to 5 grams daily. Lactulose may enhance the absorption of calcium and magnesium in foods. [7]

Leucrose

Leucrose is an isomer of sucrose, a disaccharide consisting of glucose and fructose, linked by an α-1,5 bond instead of the α-1,2 bond as in sucrose. Its relative sweetness is about 50 percent that of sucrose. Leucrose is formed as a by-product in the manufacture of dextran. In 1986 Pfeiffer & Langen was granted a patent to produce leucrose from fructose or sucrose using the enzyme dextransucrase. It has recently become an ingredient in a sweetener blend called Xtend™ Sucromalt sold by Cargill.

Tagatose
Tagatose is very similar to fructose in structure – technically it is a C-4 epimer of D-fructose. Tagatose has a physical bulk similar to sucrose and is 92% as sweet as sucrose. Tagatose provides 1.5 kcal/g and if taken in excessive quantities can cause a laxation effect. Tagatose has a minimal effect on blood glucose and insulin levels and provides a prebiotic effect. Tagatose can be considered a low-calorie, carbohydrate sweetener, which does not promote tooth decay. When using less than 3.33 g per serving of tagatose, foods and beverages in the U.S. are allowed to carry a "zero calorie" claim. When the amount does not exceed 0.5 g per serving of tagatose, foods and beverages in the U.S. are allowed to carry a "sugar free" claim. Tagatose is made by a two-stage process in which lactose is hydrolyzed to glucose and galactose and the galactose is then isomerized under alkaline conditions to tagatose.

One of the main features of tagatose is its ability to enhance flavor. It creates a synergistic flavor-enhancing effect in combination with high intensity sweeteners and is considered ideal for diet soft drinks. Combination with high intensity sweeteners speeds up sweetness onset and reduces bitterness. Tagatose is also claimed to enhance mint and lemon flavors and to boost creaminess and toffee flavor. Tagatose is pH stable in acidic applications. Tagatose is temperature sensitive and will brown and caramelize more rapidly than sucrose.

Trehalose
Trehalose is naturally present in several common foods. It is half as sweet as sucrose, with the same number of calories, 4 kcal/g, and is said to be a flavor enhancer. It received GRAS status in 2000. According to the manufacturer, trehalose is a multi-functional carbohydrate that provides energy with all the functional benefits of sucrose. Its lower sweetness is perceived as an advantage for some applications. It is made by a patented two-enzyme process from corn starch (Hayashibara International) and sold by Cargill under the brand name Ascend™. Like sucrose, it is a non-reducing disaccharide. Upon hydrolysis, it yields two glucose units. Trehalose has been shown to elicit a very low insulin response and to provide sustained energy. Among its most prominent features is that it stabilizes proteins, protects and preserves the cell structure of foods, and maintains texture in foods during freeze/thaw cycles. It is also heat stable.

The Old Stand Bys

Along with sucrose, sweeteners that have been in long time use include fructose, glucose and maltose.

Fructose
Fructose is generally recognized as sweeter than sucrose, but the degree of sweetness is highly dependent on the matrix and the temperature, but is usually in the range of 1.5-1.8 . Fructose causes less of an increment in plasma glucose and insulin response and has a low glycemic index.

Glucose
Glucose is only about half as sweet as sucrose and has a glycemic index of 100.

Maltose
Maltose is a reducing disaccharide composed of two glucose units. The tendency to brown is about one-third that of glucose. It is 40-54% as sweet as sucrose. Maltose is manufactured by liquefaction of starch followed by treatment with pullulanase and ß-amylase. Maltose has a glycemic index of 105, among the highest of any food ingredient.

Table 2 lists some of the characteristics of the sugars and sugar alcohols discussed.

The "Common Natural Sweeteners"

The natural foods market often considers sucrose unacceptable, and the Whole Foods Market lists several sweeteners that they regard as the "common natural sweeteners." [6] These include **concentrated fruit juice**, **fructose**, **honey**, **maple syrup**, **brown rice syrup**, **barley malt** and **molasses**.

Honey. Honey, available in its native form or in dried mixtures, is used for its high sweetness and flavor attributes. Honey is a mixture of many different sugars, the majority being glucose and fructose, but also with small amounts of maltose and sucrose and traces of isomaltose, maltulose, turanose, erlose, melezitose, raffinose, and kestose. Honey is valued because it provides color, antimicrobial properties, antioxidants, flavoring, texture and gastrointestinal benefits (enhances bifidobacterial growth), and conveys a natural image. The claimed glycemic load of honey varies considerably, depending on the source and composition, with reported ranges between 36 to 83, although the most usual range seen is 55-61, similar to that of sucrose. Honey is considered to be sweeter than sucrose.

Brown-rice syrup and **barley malt** have been used in a number of institutional products, although their carbohydrate distributions would indicate no major differences from typical corn syrups in terms of glycemic effects. [6] Both brown-rice syrup and barley malt, although low in glucose content, contain 40+% maltose, which has a glycemic index of 105.

Concentrated fruit juices provide perceived benefits beyond sweetness, such as color, flavor, antioxidants, humectancy, binding characteristics and a "healthy" image.

The High Intensity Sweeteners

Acesulfame potassium (Acesulfame K, Ace-K) is approved for a wide number of uses in approximately 90 countries. It was granted general purpose approval in December 2003. By itself, acesulfame-K has a bitter, metallic aftertaste, but it is synergistic with a number of other sweeteners, including high intensity sweeteners, such as aspartame. Acesulfame-K is relatively heat-stable, has a sweetness level approximately 180-200 times sweeter than sucrose, with zero calories. It is sold under the brand name Sunett and is made by Nutrinova, Inc.

Alitame is an amide analog of aspartame, discovered by Pfizer, that is 2000 times as sweet as sucrose. GRAS status has been pending in the United States since 1986 and is expected soon. It has been approved in Mexico, Australia, New Zealand and China.

Aspartame has an extensive history of use in the United States, primarily in beverages. Aspartame is a dipeptide (methyl ester of aspartic acid and phenylalanine) approximately 180 to 200 times the sweetness of sucrose. It was first approved in 1981, and was approved in 1996 as a general-purpose sweetener. Aspartame decomposes under combinations of high temperature, high pH and high moisture, but its relatively long history of use, temporal profile and economics have made it the most-used high-potency sweetener in the United States.

Cyclamate was banned in the U.S. in 1970, although it is still used extensively outside the United States. The Calorie Control Council, Atlanta, and Abbott Laboratories, are pursuing the reapproval of cyclamate. Its sweetness is relatively low among the high-intensity sweeteners, being only 30 times as sweet as sucrose, but it is synergistic with a wide array of sweeteners and polyols.

Neotame has the highest intensity of any approved sweetener. It is reported as being 7,000 to 13,000 times sweeter than sucrose, with the usual figure given as 8,000 times sweeter than sucrose. It is produced by the hydrogenation of aspartame and 3,3-dimethylbutyraldehyde. It has zero calories, and is more stable than aspartame, with greater heat stability. It was approved by FDA in 2002, and sales have risen rapidly. Neotame is made by NutraSweet.

Saccharin was the first low-calorie sweetener used. Saccharin is an extremely stable, very economical sweetener, approximately 300 times the sweetness of sucrose, and is probably most known for its use as a tabletop sweetener.

Sucralose, approved by FDA in 1998, is derived from sucrose (tri-chloro-galacto-sucrose) and is about 600 times the sweetness of sucrose. Sucralose is extremely heat-stable.

Table 3 summarizes the information about the high intensity sweeteners. Appendix 1 lists some of the brand names of various sweeteners. It is not an exhaustive list.

Other Prospective Low-Calorie Sweeteners

In addition to all of the above sweeteners, there are a number of additional prospective low-calorie sweeteners derived from plant extracts.

Dihydrochalcones (DHCs). Non-caloric sweeteners derived from the bioflavonoids of citrus fruits. Approximately 300 to 2,000 times sweeter than sucrose. They exhibit delayed sweet taste and a licorice aftertaste. Currently "Neo-DHC," about 1,500 times sweeter than sucrose, synthesized from Seville oranges, has the greatest potential for food applications. It has potential use in chewing gum, candies, mouthwash, toothpaste, some fruit juices and some pharmaceuticals. It is approved for use in the European Union and Zimbabwe, and approved in the U.S. for flavoring products such as baked goods, beverages, chewing gum, frozen dairy products, candy and sauces.

Glycyrrhizin. A non-caloric extract of licorice root, 50 to 100 times sweeter than sucrose. It is used as a flavoring for tobacco, pharmaceuticals and some confectionery products, and as a foaming agent in some non-alcoholic beverages. Its pronounced licorice flavor limits wide-spread use as a sweetener. It has been approved for use in the U.S. as a flavor and flavor enhancer.

Stevioside. It is extracted from the leaves of a South American plant, 300 times sweeter than sucrose, with a long-lasting sweet taste; stable and soluble. Used in soft drinks, chewing gum, tabletop sweeteners, fish sauces, syrups and pharmaceuticals. It has been approved for use in 10 countries, including Japan, Paraguay and Brazil, and may be sold in the U.S. as a dietary supplement but not as a sweetener or food additive. In 2004, the Joint Expert Committee on Food Additives of the World Health Organization reviewed stevioside and granted a temporary 2 mg/kg body weight Acceptable Daily Intake (ADI) for steviol glycosides. Stevia is a mix of several glycosides, some of which are sweet, and some of which are very bitter, so product purity is an important consideration.

Thaumatin (Talin™) A mixture of sweet-tasting proteins from a West African fruit, approximately 2,000-3,000 times sweeter than sucrose. Taste develops slowly and leaves a licorice-like aftertaste. It is not heat stable and so its use is limited to products that are not subjected to heat. It is approved for use in foods and beverages in Israel, Japan and the European Union. In the U.S. it is approved as a flavor enhancer for products such as beverages, jams and jellies, condiments, milk products, yogurt, cheese, instant coffee and tea, and chewing gum.

Prebiotics

The Calorie Control Council touts the prebiotic properties of many of the low-calorie bulk sweeteners. [4] Prebiotics are nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth or activity of beneficial bacteria in the colon. Prebiotics are not digested, or are only partially digested, by human enzymes and therefore have a low caloric value. Prebiotics may also increase the absorption of minerals from the diet.

Examples of prebiotics among the low-calorie bulk sweeteners include lactulose, lactitol, oligofructose and tagatose. These compounds tend to stimulate the growth of Lactobacillus spp. and Bifidobacterium and inhibit the growth of harmful bacteria, such as the proteolytic bacteria, Enterobacterium and Enterococcus.

Blending Sweeteners

There are a number of reasons to blend sweeteners: [6,8]
  1. Adjust the temporal profile of sweetness perception
  2. Mask off-tastes, such as bitterness and astringency
  3. Create synergies
  3. Reduce calories
  4. Impart functional characteristics

The blending of sweeteners has become quite prevalent in recent years. Sweetener combinations are chosen for specific reasons, such as to manipulate the sweetness level or for color, flavor, glycemic effects, to lower calorie content, to adjust viscosity, texture, water activity, humectancy, binding properties, crystallizing properties or freezing-point depression.[6]

For example, sucrose may be blended with the colored "natural" sweeteners, such as molasses or concentrated fruit juices to impart color and flavor to the system along with bulk and to keep the price down. High intensity sweeteners can be blended with the low intensity bulk sweeteners, such as some polyols, to boost the sweetness level, while at the same time, taking advantage of the lower calorie content, lower glycemic index and slower digestibility provided by the bulk sweetener. Control of the temporal profile of sweeteners is also an important reason to blend. Different sweeteners reach their maximum perceived intensity in the mouth at different times and some linger longer than others. Blends can modify or smooth out these profiles. As an example, aspartame develops maximum sweetness intensity more slowly and persists longer than acesulfame-K. By blending these two sweeteners, both the time of maximum sweetness and the "lingering" sweetness can be manipulated to be more like that of sucrose.[6] Blending of one sweetener with another can also mask the metallic, bitter or astringent off-tastes of some sweeteners, such as saccharin. HSH or corn syrup can be added to control crystallization of sucrose or maltitol.

Blending is also often done to take advantage of sweetener synergy, which occurs when the combined sweeteners are sweeter in the blend than the sum of the individual components. Known synergies exist between aspartame and acesulfame-K.

Controlled Digestibility Sweeteners

The concept of "controlled digestibility sweeteners" has been developed by Cargill. [9] The company has produced a series of slowly digestible sweeteners with physical and chemical functionalities similar to traditional nutritive sweeteners, tailored to have slow digestion rates by human intestinal enzymes and to cause a smaller rise in blood glucose and insulin levels as compared to traditional sweeteners. The sweeteners are fully digested and absorbed by the upper gastrointestinal tract. These products take advantage of the lower calorie content of certain polyols and other sugars in blends or alone, to provide sustained energy, glycemic control, and good sensory attributes.

Appendix 2 lists some current commercial blends. It is not an exhaustive list.

Summary

In summary, an array of sweeteners exists, ranging from the traditional to the novel, from polyols to high intensity, from normal caloric to zero calorie. The range is increased when sweeteners are blended to achieve certain objectives. Although many of these are designed to replace sucrose in formulas, sucrose continues to be the most widely used sweetener because few alternate sweeteners can adequately replace all of its functions.

DISCLAIMER

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by Sugar Processing Research Institute, Inc.

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Table 2. Properties of Sweeteners - Sweetener Information Table

Sweetener	Sweetness	Cal/g	GI*	Type	Source
Erythritol	0.7	0.2	0	Sugar alcohol	Fermentation of glucose by *Moniliella pollinis*, a fungus
Glucose	0.5	4.0	100	Monosaccharide	Hydrolyzed starch
Fructose	1.5-1.8	4.0	19-23	Monosaccharide	Enzymatically isomerized glucose
HFCS	1-1.2	4.0	60-65	Mixed glc/fru	Hydrolysis of corn starch and isomerization of glucose
HSH	0.5-0.7	2-4	varies	Mixed polyols	Hydrogenated partially hydrolyzed starch
Isomalt/ Isomaltitol/ Palatinit™	0.45-0.65	2.0	2	Sugar alcohol	Hydrogenated isomaltulose; equal mixture of gluco-sorbitol and gluco-mannitol
Isomaltulose/ Palatinose™	0.3-0.4	2.0	32	Disaccharide	Enzymatic isomerization of sucrose with *Protoaminobacter rubrum*; GRAS March 2006; a sucrose isomer
Lactitol	0.35-0.4	2.4	6	Sugar alcohol	Hydrogenated lactose
Lactose	0.2-0.4	4.0	46	Disaccharide	Milk sugar
Lactulose	0.6	0.2	0	Disaccharide	Alkaline isomerization of lactose when milk is heated; prebiotic
Leucrose	0.5	2.0		Disaccharide	Dextransucrase action on sucrose and fructose; dextran by-product; a sucrose isomer
Maltitol	0.5-0.9	3.0	35-52	Sugar alcohol	Catalytic hydrogenation of high maltose corn syrup
Maltose	0.4	4.0	105	Disaccahride	Enzymatic hydrolysis of starch; long time use
Maltulose	0.3-0.42			Disaccharide	Alkaline isomerization of maltose; a sucrose isomer; little used
Mannitol	0.5-0.72	1.6	0	Sugar alcohol	Hydrogenation of invert or fructose; new fermentation process
Sorbitol	0.6	2.6	9	Sugar alcohol	Hydrogenated glucose
Sucrose	1.0	4.0	61-65	Disaccharide	Cane and beet
Tagatose	0.92	1.5	0	Galactose isomer	Hydrolyzed lactose; galactose converted by alkali; GRAS 2001

Trehalose	0.5-0.7	3.6	45-50	Disaccharide	Patented 2-enzyme process from corn starch; GRAS 2000
Trehalulose	0.5-0.7			Disaccharide	Sucrose isomer; by-product of palatinose production
Xylitol	1.0	3.0	7-13	Sugar alcohol	Hydrogenated xylose

*GI = Glycemic Index

Table 3. Properties of High Intensity Sweeteners*

Sweetener	Other Names	Sweetness**	Comments
Acesulfame K	Ace K; Sunette; Sweet & Safe; SweetOne	200	N-sulfonyl amide structure; approved 2003.
Alitame	-	2000	Aspartame amide analog (GRAS pending since 1986); limited approval in 4 countries (Mexico, Australia, New Zealand, China)
Aspartame	NutraSweet; Equal	180-200	Aspartyl-phenylalanine methyl ester; approved 1981.
Cyclamate	Sucaryl, Sugar Twin	30-50	Sulfamic acid Na or Ca salt; approved in 50 countries; not USA
Neotame	-	7,000-13,000 Avg 8,000	Derivative of aspartame, more stable than aspartame; approved 2002
Saccharin	Sweet 'n' Low	300	N-sulfonyl amide structure; the first low-cal sweetener
Sucralose	Splenda	600	Trichlorinated derivative of sucrose; approved 1998.

* Cal/g = 0

** Sucrose = 1 (Relative to a 10% sucrose solution) Different numbers indicate effect in different foods

APPENDIX 1

A partial list of named sweetener products. (This is not an exhaustive list.)

Aclame™ - alitame Pfizer, Inc.
Ascend™ - trehalose, Cargill Health & Food Technology Unit
Eridex™ - erythritol, Cargill
Equal - aspartame
Finlac™ DC - directly compressible grade of lactitol, Danisco
Frutalose™ - oligosaccharide inulin syrup made from chicory root, Sensus America
Gaio® tagatose - SweetGredients, a joint venture of Arla Foods Ingredients and Nordzücker
Krystar® - crystalline fructose - Staley
Lacty® - lactitol - Danisco
Maltidex HP - Cargill high purity liquid maltitol
Maltisorb® - crystalline maltitol - Roquette
Maltisweet - crystalline maltitol products from SPI Polyols
Naturlose™ - tagatose made by Spherix, Inc
NutraSweet® - aspartame, NutraSweet Company
Palatinit™ - isomaltitol, isomalt - product of Palatinit GmbH/Group Südzucker
Palatinose™ - isomaltulose - product of Palatinit GmbH/Group Südzucker
Splenda - sucralose, product of Tate & Lyle
Sunett - acesulfame potassium
Sweet'N Low - saccharin
Sweet One - acesulfame potassium
Xtend isomaltulose - Cargill, a granular product designed to offer similar benefits to sucromalt.
Xylitol - Danisco

APPENDIX 2

Some commercial blends. (This is not an exhaustive list.)

- **Light Cane Sugar** - Tate & Lyle (2004); mixture of sucrose + sucralose (Splenda) - 33% fewer calories (11 kcal/tsp instead of 16).
- **Maltotame™** - Maltodextrin + neotame; non-caloric; Sweetener Solutions LLC, Savannah, Ga
- **Neo-Crystals™** - Sugar + neotame co-crystallized; replaces one-third the sucrose in a product; Sweetener Solutions LLC, Savannah, Ga
- Nutrinova Germany - Targets the development of specific sweetener blends containing Sunett (acesulfame K) with both nutritional and non-nutritional sweeteners. (ie, Sunett + sucralose blends)
- **Rebalance™** - Tate & Lyle concept: "Solution Sets" for sweetener alternatives with no sucrose, containing polyols and fructose. Liquid blends with low calorie bulking agents and fructose.
- Roquette Company sells maltitol as "an invaluable complement to intense sweeteners."
- **Shugr™** , Swiss Research, Health Sciences Group, a sugar substitute consisting of a blend of erythritol + tagatose, also containing maltodextrin and sucralose. The ingredients carry GRAS designation and the company claims that the inclusion of tagatose, which acts as a pre-biotic and a fiber, may also allow some health claims. Available in health food and natural products stores. Said to have zero calories and to cook and taste like sugar.
- **Sorbitame™** - Sorbitol + neotame, Sweetener Solutions LLC, Savannah, GA
- **Splenda Brown Sugar Blend** - Reduced calorie brown sugar - McNeil Nutritionals/Johnson & Johnson
- **SucraSweet™** - Neotame + acesulfame K + maltitol, Sweetener Solutions LLC
- **SweetDesign™** - Cargill - a variety of sweeteners blended to reduce calorie and sugar content. Products include a variety of sweetening, stabilizing and texturing ingredients, including erythritol
- Tate & Lyle flavored water - splenda + fructose
- **Twinsweet™** - Blend of aspartame + acesulfame K, linked together as an "ionic salt"; no calories - Holland Sweetener Co. Twinsweet™, a salt of aspartame and acesulfame linked at the molecular level (64% aspartame and 36% acesulfame). It has approximately 300 to 400 times the sweetness of sucrose, and reportedly eliminates the off-taste associated with the potassium salt of acesulfame.
- **Xtend™ sucromalt**, Cargill, described as "a syrup derived from sucrose and maltose through an enzymatic process." Composition (from company literature): 37% fructose, 13% leucrose, 48% higher saccharides (35% resistant maltodextrin); a slowly digestible sweetener 70% as sweet as sucrose, a non-crystallizing syrup.
- Sweetener Solutions LLC produces a number of sweetener blends containing neotame blended with maltitol, maltodextrin, or sorbitol, among other combinations. Their brands include **SucraSweet, Maltotame,** and **Sorbitame**, listed above.

Food Trends for a New Century

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Abstract

In the last decade, large sugar companies worldwide have increasingly become diverse ingredient suppliers. While in many cases, sugar remains the main business focus, acquisition of ingredient manufacturing companies and new product development continue to widen the portfolio of many sugar companies. With that in mind, it is worthwhile to consider some emerging food trends that impact ingredient companies. A year-long examination of headlines in the daily online news outlet, Food Navigator, revealed six major areas that currently are driving changes in the food industry: Environmental Issues; Fears and Controversies; New Technologies; The Consumer; Functional Foods; and By-Product Value. This paper briefly highlights several pertinent issues within each of the identified trend categories. Examples that illustrate each category are presented.

Food Trends in the Twenty-First Century

Food trends can be categorized in different ways. It is important for today's sugar industry and sugar based products industries to address such issues because the key to successful product development is the ability to recognize and exploit trends. According to food expert, Phil Lempert, (Food Navigator, 05/05/2006) a better understanding of consumer trends is essential if the food world is to realize its opportunity and potential. He says that firms must focus on aging consumers, ethnic diversity and time constraints. It is not possible to have one product that appeals to all consumers; products need to be geared to many different types of consumers and their needs.

In the study reported here, the daily online news outlet for the food industry, Food Navigator, was used as the source of information for current important trends impacting the food industry. These issues were noted to come up time and again over the year and were noted to fall into six general categories: Environmental Issues, Food Safety Fears and Controversies, New Technologies, The Consumer, Functional Foods, and By-Product Value.

Environmental Issues

Environmental issues pose real challenges and dangers to the food industry, and both industry and consumers are concerned about the potential for major future problems if these are not addressed in a timely manner. According to Michael Grier (Corporate Social Responsibility Manager at Tate & Lyle - Food Navigator, 02/24/2006), good environmental management is integral to good overall management of business.

Below are a few headlines that highlight some of the environmental issues facing the food industry in the Twenty-First Century.

Soil erosion could devastate food sector. (03/23/2006) Soil erosion is threatening the future of food production, according to a new Cornell University study. Valuable earth is being washed away 10 to 40 times faster than it is being replenished, adding to the growing body of scientific evidence that environmental change is having a direct negative effect on the food sector. "Soil erosion is second only to population growth as the biggest environmental problem the world faces," said David Pimentel, professor of ecology at Cornell. "Yet, the problem, which is growing ever more critical, is being ignored because who gets excited about dirt?"

Food industry anxious over rising rapeseed demand for biodiesel. (04/05/2006) This headline highlights the growing "food vs fuel" debate. Rapeseed oil, for general food use, has become the focus of heated debate between the food sector and biodiesel advocates. Biofuel producers blend the oil with conventional diesel to provide a greener alternative for the EU transport sector. Good intentions to limit dependence on imported oil and to reduce emission of high levels of greenhouse gases have, at the same time, led to concern that the food industry will suffer as it competes for limited resources of rapeseed. Already, up to 58 per cent of total rapeseed oil made in the EU 25 countries is used for biofuel.

Another source of biofuel is, of course, ethanol, increasingly produced from sugar and corn, so similar concerns about competition for these commodities as either food or fuel arises, with the specter of increased prices and scarcity.

European food giants to shun illegal Amazon soy. (07/24/2006) Europe's leading food manufacturers and retailers were expected to announce they planned to shun soy grown illegally in the Amazon, which is resulting in depletion of the rainforest. This headline highlights the growing awareness of environmental devastation that can occur with ill-considered exploitation of the land.

Pharmaceuticals in drinking water. In May 2006, at the International Society of Beverage Technologists (ISBT), Eric G. Isacoff, Senior Consultant, Heley & Aldrich, gave a presentation entitled "Analysis, occurrence and potential remediation of low levels of pharmaceutical products/byproducts in drinking water."

Food Safety: Fears and Controversies

There is no end to the "fear of food" these days. Headline after headline demonstrates it: Bird flu, BSE, pesticide residues in food, benzene in beverages, increasing food borne pathogens, new pathogens emerging, increased food allergies, "junk food" in schools, GM (genetically modified) food, "bad" ingredients in food, banning certain foods, ascendancy of organic. These are real issues that , while often exaggerated by interested parties, do need to be addressed, so that underlying trust in the food supply can be maintained.

The basic problem, of course, is that the consumer has begun to question the safety of the food supply. This is more evident in some countries than in others. For example, studies of American consumers show them to be more trusting of their food supply than consumers in Europe. This may be due to a strong underlying trust of the government agencies responsible for food safety, such as the Food and Drug Administration (FDA), U.S. Department of Agriculture (USDA) and the Environmental Protection Agency (EPA). Such trust is precious and should be preserved because when trust is breached, it is difficult to regain.

Fears about the safety of any food can lead to incalculable losses in revenue when the produce of a whole area or of a country is banned from trade to other countries, or when a company's product comes under question.

Animal diseases. The emergence of frightening animal diseases that can be transmitted to humans has led to tremendous changes in global trade. As an example, Japan, the largest buyer of U.S. beef, instantly dropped the U.S. upon the discovery of one animal with BSE and switched to Australian beef. The report of one affected cow can bring the beef industry to a complete halt in an area and result in the slaughter and destruction of many animals. Worldwide, the consumption of beef has decreased. New rules regarding age at slaughter and type of feed are helping to alleviate some of the concerns and apparently has halted the spread of "mad cow disease."

The emergence of avian influenza, or bird flu, has led to the slaughter of millions upon millions of birds worldwide, and complete stoppage of trade between long time trading partners. The march of avian flu across countries is watched with dread. Poultry consumption has plunged by up to 70 per cent in some countries.

Benzene in beverages. The issue of benzene in certain soft drinks arose several years ago. Two common beverage ingredients, ascorbic acid and potassium benzoate, can produce benzene, a carcinogen, when exposed to heat and light. The perceived failure of companies to address this problem publically has led to continued problems of perception, although soft drinks have been reformulated and the problem is essentially solved.

Pathogen contamination. Repeated reports of the contamination of fresh produce with Salmonella is leading to fears about eating fresh produce. In fact, fresh produce is catching up with chicken as a major culprit of Salmonella infections in the US, according to an analysis of food-poisoning outbreaks by a consumer lobby group. The most notorious recent example was the contamination of spinach in the U.S., when the sale of fresh spinach came almost to a complete stop.

Food allergens - The "free-from" trend. The "free-from" food market has been growing rapidly. This market sector includes foods that are free from such ingredients as dairy, gluten, wheat, nuts, etc., that cause serious food allergies in a growing number of individuals. In the U.K., this sector has grown over 300 percent since 2000 and is set to double (Julie Sloan, Mintel Market Analyst, 03/22/2006). Even people who do not suffer allergies or food intolerance are enjoying free-from diets as a trendy lifestyle choice.

Banning certain foods as "BAD." Many schools in the U.S. have begun to remove or restrict the sale of snack foods and soda, calling them "junk food" that is bad for children. Certainly ingredients, such as sugar and fats, have suffered from these negative perceptions over the years. In December 2006, restaurants in New York banned trans-fats.

Continued global split over GM food. Genetically modified crops, also known as biotech crops, have been around for several decades, but the argument about their safety and acceptance continues unabated. There is a stark delineation between their general acceptance in North America, especially in the U.S., and their near total rejection in Europe. The science of the issue hardly seems to matter, as positions have hardened and emotions are engaged. With the approval of biotech sugarbeets and the expected advent soon of biotech sugarcane, this is an issue of vital interest to the sugar and confectionery industries. Decisions about using these types of ingredients will have significant repercussions on trade.

Organic is better. Organic is safer. Organic is more nutritious! The organic food market has been growing at a rate of about 24 percent per year for over ten years. Organic foods are very attractive to consumers who perceive that the normal food supply is somehow not safe or nutritious or may be genetically modified. As organic food production moves into the mass market, it becomes more difficult to maintain the stringent parameters laid down for organic certification, and there is no doubt that organic farming as practiced today cannot sustain the world's population.

Removal of artificial ("non-natural") ingredients in favor of "natural" for a clean label. With stronger labeling requirements these days, many food companies prefer to have what is known as a "clean label" - that is, a label that avoids the use of artificial ingredients or long chemical names that might frighten away a potential consumer. This trend has led to an explosion of creative food science in search of natural substitutes for functionalities previously contributed by "chemicals."

As an example, in May 11, 2006, it was reported that Nestlé had removed artificial colors from its Smarties brand of confectionery. The company said it was pursuing a consumer health trend, and was expecting to attract parents concerned about their children's intake of certain foods. Unfortunately, the blue Smartie will no longer exist, as the company was unable to find a suitable natural alternative.

In another example, it was reported in April 26, 2006, that Chr Hansen had developed a natural meat curing ingredient that could be used in place of added nitrite, helping food makers to develop clean-label meat products. The company claimed that the new ingredient creates the necessary color and flavor, and offers the same functionality as traditional curing methods.

New ingredients are driving sugar-free development. The use of ingredients to improve the nutritional status of food products by replacing sugar is one of the major driving forces for new

product development. Many new polyol sweeteners and combinations have come on the market, led by companies such as Roquette and Cargill. Food makers have a strong incentive to remove sugar from the label because "sugar free" is perceived as healthier. This topic is covered in greater detail elsewhere in this volume (pp. 84-98).

Technology

Advances in technology make new products and processes possible. Several interesting technologies were reported or expanded in 2005/2006.

Nanotechnology - A Truly New Thing. Nanotechnology is a new concept that is not yet fully understood, and is looking for applications. There is a sense in the food industry that it will soon become useful, especially in packaging. As of March 20, 2006, fifteen food and drink items were listed in a new online nanotechnology database.

New chip targets improved citrus flavor. (THIS IS NOT A POTATO CHIP) (03/03/2006) Scientists have developed a chip that can improve the flavor of citrus varieties, which could have consequences for the growing natural flavors sector. By helping determine which genes are turned on in a tissue of citrus genes that are associated with taste, acidic content and disease, the chip, called the GeneChip Citrus Genome Array, could provide information useful to researchers for rectifying existing problems and making improvements to the fruit. "The citrus array helps us quickly examine a certain trait in citrus," said Mikeal Roose, a professor of genetics in the department of botany and plant sciences at University of California Riverside and a leader of the three-year research project. "For a trait posing a problem for the consumer, such as an undesirable flavor, we can identify genes associated with the trait and target these for correction to improve the flavor."

Genomics: Genomics has the potential to revolutionize food science. (04/09/2006) Many scientists believe that genomic technology has the potential to alleviate food insecurity and food shortages around the world. Biotechnology, for example, has the potential to improve the nutritional content of food crops and resistance to insects and disease, leading to improved yields of food crops. Researchers are also working on "molecular farming."

"Nutrigenomics" - Personalized Nutrition. (03/03/2006) Personalized nutrition has been identified as an "emerging business area." The "one-size-fits-all" approach to nutrition is no longer seen as fulfilling the needs of an increasingly health conscious and ageing population that is determined to remain healthy longer. Nutrigenomics is a scientific term coined in 1999 that involves the development of new nutrients and understanding of how they work in the human body. Nutrigenomics is the study of how nutrients and genes interact and how genetic variations can cause people to respond differently to food nutrients. Nutrigenomics is still in its infancy, but scientists predict that this branch of study has the potential to bring about radical changes in how food is grown, processed and consumed, and it could lead to personalized diets tailored to the genetic make-up of the individual.

New ways to kill pathogens without heat. Scientists are studying the effectiveness of new methods, such as ozone, supercritical carbon dioxide, electrolyzed oxidizing water and pulsed UV-light, to kill

pathogens without using thermal processes. More effective sanitizing processes that do not use chemicals or heat are attractive to processors, not only to lower costs and disposal problems, but also because heat processes often reduce the quality of foods. Other emerging sanitation technologies include irradiation, high hydrostatic pressure, pulsed electric field and ohmic heating.

Natural antimicrobial market boosted by processing trends. (07/21/2006) The introduction of more additive-free foods is boosting the use of natural antimicrobials in almost all food processing segments. At the same time, the "clean label" emphasis hinders the growth of the synthetic antimicrobials market, so, in addition to exploring the use of newer processing and preservation technologies, such as ohmic heating, high-pressure, pulsed electric field, bright light, and aseptic processing that can limit the application of synthetic antimicrobials, these trends are also creating new market opportunities for natural antimicrobials, such as nicin and natamycin, whose application scope is gradually extending to areas such as bakery products, meat, and convenience food products.

Robotics: The future of food processing? (05/04/2006) A recent food show featured the future of food processing in a working sausage processing and packing line set up by robot manufacturers. Meat went into a hopper at one end and came out the other palleted and wrapped for transport, all with minimal human intervention. Robotics could begin to take a more prominent place in the arsenal of options available to food manufacturers when they consider how to automate more of their processes. Robotics holds out the promise of reducing costs by helping to speed up lines, making production more efficient and reducing labor requirements.

Robotics for Danisco enzyme and culture screening. (01/14/2006) Making use of state-of-the-art technology within robotics, food ingredients company Danisco has strengthened its biotechnology capacity in enzymes. The robotic system enhances the company's capacity to find and isolate new enzymes and cultures. States Andrew Morgan, Scientific Director, "In nature, there is constant evolution. We capitalize on that naturally-occurring process in the laboratory, simply accelerating and guiding the process to identify or design enzymes and cultures with the properties needed by our customers." However, identifying the enzyme that possesses exactly those desired properties can be a complicated and lengthy process if done manually. Perhaps one million variants would need to be examined to find the right one. By using a robot system, more sophisticated identification methods can be used to screen variants more efficiently. With a robot system the company is able to screen over 20,000 variants per day, whereas manually only a few hundred can be checked. The robot system has the potential to provide further options for development of other ingredients; for example, in the area of flavors.

Enzyme Technology: Enzymes can tackle trans fats, claims Novozymes. (04/25/2006) In another enzyme application, Novozymes disclosed a new enzyme technology that offers the possibility of obtaining necessary melting properties without producing harmful trans fats. The company has developed an enzymatic inter-esterification process, which uses enzymes to develop healthier fats and oils. The company claims that this process is a viable alternative to the conventional partial hydrogenation technique that produces large amounts of trans fats. Chemical inter-esterification, another method of adjusting the melting point of fats, has unwanted side effects – it has a negative impact on the environment and leads to discoloration of the fat, requiring further processing. Using enzyme technology means that trans fats are avoided, the environment is spared, and a natural product is obtained with natural flavors and the desired melting properties.

Molecular Physics: Nestlé probes molecular physics of food to protect flavor, nutrition. (07/21/2006)   Nestlé Research Center, in collaboration with the University of Bristol, is using molecular physics to explore the properties of carbohydrates in food. An expanded understanding could lead to food formulation with improved flavor impact and nutritional value. "Increasingly detailed scientific knowledge, often at the molecular level, is needed to provide quantitative guidance for the development of innovative foods and food manufacturing processes," claimed Dr. Job Ubbink from Nestlé Research Center.

One such example of this kind of research was published in the journal Nature Materials (August 2006) which reported the molecular investigation of the mobility of water in amorphous and crystalline trehalose, a sugar found naturally in mushrooms, honey, lobster and shrimp. The research on the molecular physics of carbohydrates used Positron Annhilation Lifetime Spectroscopy (PALS) to study the free volume in trehalose, and showed that changes in this free volume are directly connected with molecular structure and mobility of water in the crystalline and amorphous states. "In the case of the barrier properties of amorphous carbohydrate-based foods, this is relatively easy to explain," said Dr. Ubbink. "The more dense we pack the carbohydrate molecules together, the more difficult it is for a permeating molecule (for instance oxygen), to diffuse through the food matrix and then oxidize sensitive compounds." And so by optimizing the molecular packing of carbohydrates, it is possible to influence and reduce the rate of oxidation of food ingredients such as flavors and polyunsaturated fatty acids.

The Consumer

In the final analysis, the consumer is the most important link in the food manufacturing chain, since it is the consumer who makes the decisions about what to buy and what not to buy. These days, the consumer has a lot of issues to deal with.

Confused Consumers
Increasingly complex nutritional information is being offered to the consumer, who is faced with competing studies on the efficacy or non-efficacy of certain nutrients. A study (02/24/2006) showed that most Americans are unaware of the nation's new dietary guidelines; at the same time, a strong majority said they would like to see food companies offer more health tips on their product labels. The survey also revealed that most consumers are not eating the recommended daily amount of fruit and vegetables. Even though the diets of most Americans remain lacking, the desire to consume healthy foods remains intact.

Ethical Consumer
Food challenge for 2006: Satisfying the ethical consumer. (01/05/2006) Ethical considerations increasingly dictate food purchases, and companies that don't pay attention to this defining trend will suffer. Factors such as how companies source their products, effect they have on the environment and how they treat their workers are beginning to have a direct impact on how consumers spend their money. People are increasing asking where their products were made and how far they have come. A website, www.ethicalconsumer.org, has the purpose of informing and empowering consumers to enable them to make ethical purchasing decisions. The fair trade movement has also gained considerable momentum, with consumers increasingly prepared to pay more for guarantees of fair

labor practices and sustainable sourcing. Nestlé UK recently became the first of the four major coffee roasters to offer a fair trade product line. Food companies are beginning to realize that tapping into ethical consumerism makes good business sense.

No avoiding sustainable sourcing. (11/07/ 2005) Food companies do not yet face the ethical sourcing equation of the clothing industry, where brands from Nike to Marks & Spencer cannot afford a single claim of sweat-shop production, but the moment is fast approaching for food, too, when exploitative sourcing will be the public relations kiss of death. Along with Groupe Danone and Unilever, Nestlé is a founding member of the Sustainable Agriculture Initiative Platform, a 19-member food industry body which supports the development of agricultural practices that preserve current resources and enhance their efficiency.

Sustainable development a business reality, says report. (04/25,/2006) Sustainable development will steadily advance over the next 10 years according to a new PricewaterhouseCoopers report - an issue that the food industry must positively address. Sustainable development is the challenge of creating strategies to meet immediate needs without sacrificing the needs of future generations. "Sustainable businesses balance their economic interests with the need to be socially and environmentally responsible," according to Sunny Misser, PricewaterhouseCoopers global leader of sustainable business solutions. "The companies that succeed over the long term are those that integrate ethical considerations into company decision-making, and manage on the basis of personal integrity and widely-held organizational values."

Industry targets growing demand for local food. (03/22//2006) The increasing availability of locally grown ingredients and food is driving sales of products with a clear provenance, according to a new report commissioned by Food from Britain (FFB). The research showed that since March 2005, the percentage of shoppers claiming to buy local food and drink had increased by 6 per cent per year. A further 9 per cent expressed an interest in buying if availability was better. Locally produced food is increasingly tapping into the ethical consumer trend. Food campaigners point out that transport of food by air, which creates the highest CO_2 emissions per ton, is the fastest growing mode of food transport. Buying local produce can cut out these "food miles," thus helping the environment.

Certification boost for ethical food makers. (04/02/2006) New third-party verification that food has been produced sustainably could help food manufacturers tap the growing trend towards ethical consumerism. The ProTerra Certification Programme from Cert ID provides socially and environmentally responsible companies with the opportunity to obtain recognition of their practices, and to be confident that the materials they purchase have not been produced in a manner that contributes to social and environmental degradation. The program is also designed to help suppliers assure their buyers, and ultimately consumers, that their products have been produced sustainably. According to Pejling, the magazine of the Swedish Dairy Association, seventy per cent of Swedish consumers have a "personal blacklist" of products and companies that do not meet their personal standards for social and environmental responsibility. In addition, a survey by Market & Opinion Research International in the UK revealed that as many as one-fifth of the UK population boycotts or selects goods on social grounds. Similar statistics are found in many countries around the world.

Consumers drive organic food practices. (04/20/2006) British food retailers and suppliers have developed a growing commitment to domestically produced organic food, as consumers insist on more environment-friendly production practices. Recent consumer pressure to cut organic food miles has led to a greater working relationship between British retailers and farmers, as supermarkets strive to source fresh produce locally. Sainsbury's, currently the UK's third largest chain, has seen demand for British organic milk soar by 80 per cent in 2005, and now encourages domestic farmers to convert to organic production. It has promised suppliers it will contribute to organic conversion costs in a new three year scheme. Of the organic staples sold in UK supermarkets, including apples, beef, onions and potatoes, 82 per cent are sourced from domestic farmers.

Strategic Philosophy - Social Responsibility. (04/24/2006) Another new trend that hopes to win over the consumer, and related to ethical consumerism, is strategic philosophy, which is when a company "gives back" to the world through charitable donations. Often these programs involve the consumer in the sense that the more the consumer buys, the more the company gives to charity.

The Aging Consumer
Leading companies reveal plans at food summit. (10/03/2006) Leaders in the US food industry met at the Reuters Food Summit to discuss the challenges that lie ahead and to outline companies' responses to changes in the industry. Kraft Foods, Unilever and Sara Lee were among the companies that identified the need to target the nation's aging population, since consumers over 50 have more disposable income and time to spend. Initiatives, such as age-friendly packaging and new products aimed to appeal more to seniors, were mentioned.

Hectic Lifestyles and Hectic Consumers - Diet changes, more snacking and eating out: **Lifestyle positioning**: **Growing herbal beverage market.** (07/03/2006) US-based firm RushNet believes it is on to a winner with a new range of natural ginseng-based energy beverages, Ginseng Rush XXX and Rush Ginseng Cola XXX.   The beverages are "ginseng-powered" and non-caffeinated energy drinks. Beverages that rely on caffeine have to put caffeine-warning labels on their cans. Herbal juice drinks for adults, with formulations that include ginseng, guarana, echinacea and/or kava root are becoming popular in Europe and have been successful in the U.S. Claims mainly focus on "lifestyle'" positioning (e.g., energizing, stimulating, calming, relaxing) or are influenced by health (e.g., protection against colds and other common ailments).

Obese Consumer
EC food industry urges multi-factor approach to obesity. (05/04/2006) The current obesity crisis can only be tackled through a greater understanding of all the related factors and an acceptance that a lack of physical activity could be a major contributor, according to a recently published report of the European food and drink industry (CIAA). There is a definite need for greater understanding of all obesity-related factors. Fourteen million Europeans are obese or overweight, of which more than 3 million are children. Obesity-related illnesses, which include heart disease and diabetes, account for up to 7 per cent of healthcare costs in the Union. In some Member States, over a quarter of the adult population is now obese. A green paper published by the EC makes it clear that the EC considers industry self-regulation the best way of dealing with the problem. However, according to the CIAA, "Ultimately, each consumer is responsible for ensuring that his or her own lifestyle is a healthy one. Parents have a similar responsibility for their children. These individual responsibilities cannot be removed."

Vegetarian Consumer: New labeling guidance taps growing vegetarian demand. (07/04/2006) New UK guidance on food labeling for vegans and vegetarians builds on growing consumer demand for non-meat ingredients. The guidance, drawn up by the Food Standards Agency (FSA) after consultation with stakeholders including The Vegetarian Society and The Vegan Society, will provide criteria for the use of the terms "vegetarian" and "vegan" on food labels for the first time. The FSA estimates that there are 3.5 million vegetarians and 0.25 million vegans in the UK. Scares such as BSE in cattle and avian flu in poultry have prompted consumers and marketers to cast about for non-meat alternatives. Overall vegetarianism has followed a steady upward curve over the past decade. A 2002 Datamonitor report estimated that there are around 12 million vegetarians across Europe.

Functional Foods –
Continued Momentum

Functional foods and supplements continue to expand in popularity because of the perceived health benefits. Hardly a day goes by without another food compound or extract being reported to impart a beneficial new health effect. There has been a tremendous increase in reports on nutrient health benefits and attempts to capitalize commercially on studies. Among the most popular components are polyphenolics and antioxidants. Anthocyanidins and procyanidins are polyphenolic compounds found naturally in olives, green tea, fruit, vegetables and red wine, which have been linked to a wide range of health benefits, including reduced risks of cardiovascular disease and certain cancers. Below is just a smattering of the sort of research headlines found in FoodNavigator.com.

Ginger may prevent diabetic kidney damage – animal study. (10/03/2006) Ginger could help protect against kidney damage, a condition said to threaten one in three diabetics.

Vitamin K1-rich diet linked to better heart health. (08/29/2006) A high daily intake of vitamin K1, found in green leafy vegetables, could reduce the risk of fatal coronary heart disease by 19 per cent, says a new study.

Cholesterol-lowering action of plant sterol-enriched products. Plant sterols are naturally occurring compounds that interfere with cholesterol absorption and thus reduce blood cholesterol levels. Although the cholesterol-lowering efficacy of plant sterols is well acknowledged, additional studies are needed to determine whether dietary cholesterol levels affect the cholesterol-lowering action of plant sterols, to establish the best time of day to consume plant sterol-enriched products, and to assess the optimal dose frequency of plant sterol intake. Fat spreads enriched with plant sterols/stanols have been shown to be effective in reducing circulating cholesterol levels in healthy adults with both normal and high cholesterol levels, as well as in children with hypercholesterolaemia.

Pomegranate juice shows possible diabetes benefits. (08/28/2006) A new Israeli study suggests that pomegranate juice could offer health benefits for diabetics.

Low GI diet could protect eyes against AMD. (07/04/2006) Eating a low glycemic index (GI) diet could reduce the risk of the incurable eye disease, age-related macular degeneration (AMD), by more than 60 per cent, says a new study. Researchers from Tufts and Harvard Universities report that,

although they observed an association between AMD and low GI, low GI diets are frequently richer in micronutrients essential for eye health that were not accounted for in their analysis. When the researcher looked at the relationship between total carbohydrate intake and the risk of age-related maculopathy (ARM) they found that a high GI diet was associated with a 50 per cent increased risk.

Pharmachem launches carb blocker for food use. (04/26/2006) Pharmachem Laboratories has introduced a new ingredient, called StarchLite, an extract of the common white bean (*Phaseolus vulgaris*) that lowers the calorie-count and glycemic index of starchy foods. It works by binding temporarily to alpha-amylase, the enzyme responsible for digesting complex carbohydrates, thereby delaying the absorption of carbohydrates and potentially promoting weight loss. Study results released earlier this year showed that StarchLite has the potential to significantly reduce the glycemic index of white bread by 20.23 points, or 39.07 percent. It is suitable for use in all food and beverage products that normally have a heavy carbohydrate load, including cereals, frozen foods, packaged meals, pasta, pizza crust, soups and confectionary.

Replacing Synthetics: Plant extracts beat synthetics as meat preservatives, says study. (09/11/2006) Grape seed and pine bark extracts used as additives in cooked meats performed better than the synthetic preservatives in oxidation and microbial effects, results that could be readily acceptable to consumers seeking ready-to-eat meat products with natural preservatives.

Red cabbage could cut Alzehimer's risk. (10/03/2006) In a new study, red cabbage was seen to reduce the build-up of certain plaques in the brain that could cause Alzheimer's. The higher concentrations of antioxidants, particularly the anthocyanins, from the red cabbage resulted in greater protection from the A-beta-induced toxicity to the cell cultures. White cabbage showed lower activity.

A similar report in the Journal of the Science of Food and Agriculture reported that blackcurrant extracts provided a protective effect against Alzehimer's in vitro. The protective effects of the currants were linked to the high anthocyanin content.

Tea's brain health benefit link gets more support. (03/31/2006) Both green and black tea could protect against age-related diseases like Alzheimer's, says a new study, adding yet more support to the benefits of tea extract on brain health. The catechins in the extracts had a protective effect on dying nerve cells.

Results from FoodNavigator.com for epigallocatechin gallate (EGCG) in green tea. A search of FoodNavigator.com for green tea reported many research studies that showed the potential health benefits of green tea and the chemical epigallocatechin gallate, found in green tea extracts: Protects against Alzheimer's (03/01/2006); reduces the risk of breast cancer (02/21/2006); enhances metabolism safely (07/25/06); able to block bladder cancer cells (02/16/05); good for skin health (10/14/2006); fights liver damage in mice (09/07/04); fights flab (06/04/2004); may prevent cancer of the esophagus (05/25/2004); stops the progression of cancer (03/29/2004); helps control allergies (09/23/2002).

By-Product Value
Mining Food Processing Residues for Value

A particularly interesting trend is the increasing number of new products with useful functions that have been developed from hitherto waste processing streams from food production. A few of these are highlighted here.

Residues from star fruit could offer inexpensive source of antioxidants. (03/07/2006) Residues from the juicing process of star fruit is a rich source of extractable antioxidants. Also known as carambola, star fruit is grown extensively in Southeast Asia, Australia, South America, Hawaii and Southern Florida.

Z-Trim Plus claims double-edged health benefits. (03/29/2006) Z-Trim Plus, launched by FiberGel Technologies is a new line of fat substitutes made from the hulls of corn, oats, soy, rice and barley which claims to be able to cut up to half the fat content of products while fortifying them with healthy fiber ingredients.

More support for grape seed extract's health benefits. (03/21/2006) Oligomeric proanythocyanidins in grape seed extracts are claimed to reduce free radical damage of blood vessel cells by 85 per cent and to protect against heart disease. *Vitis vinifera* seed extract is a rich source of antioxidants such as catechins and 2,5-flavan-3-ols.

Tea waste rich with extractable antioxidants. (02/12/2005) Tea waste is almost as rich in potent antioxidants, such as catechins, as the new and expensive green tea leaves used by the supplements industry, according to recent research. Tea leaves that have already matured on the plant and the waste that is left after fermenting black tea are often considered as agricultural wastes, but they could be used as potent natural antioxidative sources. With tea prices at around $1.50 per kg, the possibility of cheaper raw materials could shift the industry's cost base. Green tea and green tea extracts are currently produced from only the first two to four leaves of the tea plant (*Camellia sinensis*). The other leaves are known as old tea leaves (OTL). Fermenting the green tea leaves produces black tea, and produces black tea waste (BTW). Both OTL and BTW produce high extraction yields of polyphenols, including epigallocatechin gallate (EGCG), one of the most widely researched polyphenols in green tea.

Fruit and vegetable waste rich in extractable antioxidants. (03/15/2006) Fruit and vegetable waste products offer a cheap and practical source of antioxidants that could be used as functional ingredients. A recent study looked at the practical, economic, and industrial viability of waste products from juice production, waste from the canning industry, and remains from harvesting for 11 different fruits and vegetables. Initial screening of red beet, apple, strawberry and pear residues from juice production; tomato, artichoke and asparagus from the canning industry; chicory, endive, cucumber and broccoli remains from harvesting; and golden rod herb and woad herb extracts showed that all of the wastes yielded polyphenols. The high water content of the fresh byproducts required a cost-intensive drying process, and the study proposed that drying should be performed close to the production site.

Olive antioxidant from olive processing waste water. (06/29/2004) Spanish functional oils company Genosa launched a new line of products based on a highly concentrated form of the olive polyphenol, hydroxytyrosol. The ingredient can be added to a variety of different foods to boost their antioxidant and disease-fighting potential. Hydroxytyrosol is thought to be the main antioxidant compound in olives, and believed to play a significant role in the many health benefits attributed to olive oil. Research by a team from the University of Barcelona found that LDL or "bad" cholesterol levels could be cut substantially after consuming just 25 ml of virgin olive oil daily for one week. Other studies have suggested that it could also protect against cancer.

Carrot juice waste may be a new functional fiber source. (04/18/2006) The solid waste from carrot juice production is rich in insoluble fiber and may reduce cholesterol levels. The fiber-rich pomace is available in large quantity in carrot juice production.

Cider by-product provides natural alternative to tartrazine. (10/04/2006) French researchers say a by-product of the cider industry could provide an alternative to synthetic colorants such as tartrazine. The food and cosmetics industries have few choices when they are looking for a water-soluble yellow colorant. One of the most widely employed is a synthetic pigment, tartrazine, which is suspected of causing asthma and urticaria. Tartrazine is banned in several European countries. The French researchers studied the natural yellow pigment, phloridzine oxidation product (POP), which has antioxidant properties and is obtained from apples. Phloridzine is a polyphenol specific to apples. When apples are pressed to obtain juice, phloridzine, oxygen and polyphenoloxidase enzyme combine to form POP. The purified POP pigment produces a brilliant yellow color, which is freely soluble in water and stable over a wide pH range.

Peanut waste to rival grape seed extracts? (04/24/2006) Peanut skin, a waste product from peanut butter manufacturers, currently used as animal feed, is a rich source of extractable procyanidins and could create a cheaper source of these antioxidant compounds to rival the grape seed dominated polyphenols market. Peanut skins are a rich source of oligomeric proanythocyanidins – dimers, trimers and tetramers of procyanidin monomers, and could provide an inexpensive source of natural antioxidants, such as catechins and procyanidin, for use in food and dietary supplements.

Chocolate-guzzling bacteria emit hydrogen. (07/31/2006) Confectioners could soon find themselves helping the environment following the news that scientists have found a way to extract hydrogen from chocolate waste. University of Birmingham scientists released bacteria into high sugar waste and found that they emitted hydrogen gas as they consumed the substance. The discovery could have useful implications for the confectionery industry which currently disposes of waste on landfill sites but could now make use of their waste by-products as a source of environmentally-friendly energy.

In summary, many current food trends can impact the sugar industry and can point to ways in which to increase by-product value or to extend into other areas of food technology as ingredient suppliers.

The Direct Production of White Sugar in a Cane Sugar Mill

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Abstract

A new process for the production of white sugar in a cane sugar mill has been developed. It has been tested in the laboratory and during two seasons on a small scale in a Louisiana sugar mill. The process does not require membrane separation and involves adsorption of color and other impurities using granular activated carbon and ion exchange resins. Chemical regeneration of the carbon is utilized, which enhances the attractiveness of the process. The paper describes laboratory work to characterize the adsorption properties and kinetics and a comparison with pilot plant data. Preliminary information is given on a possible full-scale installation in a raw sugar mill.

Introduction

Some significant trends are evident in the world sugar market. In the 1980s the proportion of white sugar traded on the world markets, both beet and cane showed a clear increase relative to the raw sugar (*Clarke,* 1986). More recently, the trend has changed, since a number of new large refineries have been built and white sugar exports from producing countries, particularly from the EU, have reduced. Another trend evident is the swing to VHP or better raw sugar supply to refineries, and in particular an increase in very low color raws exemplified by Brazilian "cristal".

A further trend evident in the US has been the increase in vertical integration from field to white sugar output. All beet sugar factories in the US are now owned by the growers, and many large raw cane sugar producers have invested in refining, both white-end refineries and in existing or new refinery capacity.

These trends illustrate the interest shown in producing better quality sugar. There is therefore considerable value in attempts to produce cane white sugar directly, or at least to produce a very high quality and low color raw sugar.

The raw sugar produced in a cane sugar mill is normally sent to a refinery, to be converted from a light brown raw sugar with a purity of around 98.5% to an almost pure (>99.9%) white sugar for sale to consumers. A flow diagram showing the unit operations in a raw sugar mill is shown in Figure 1.
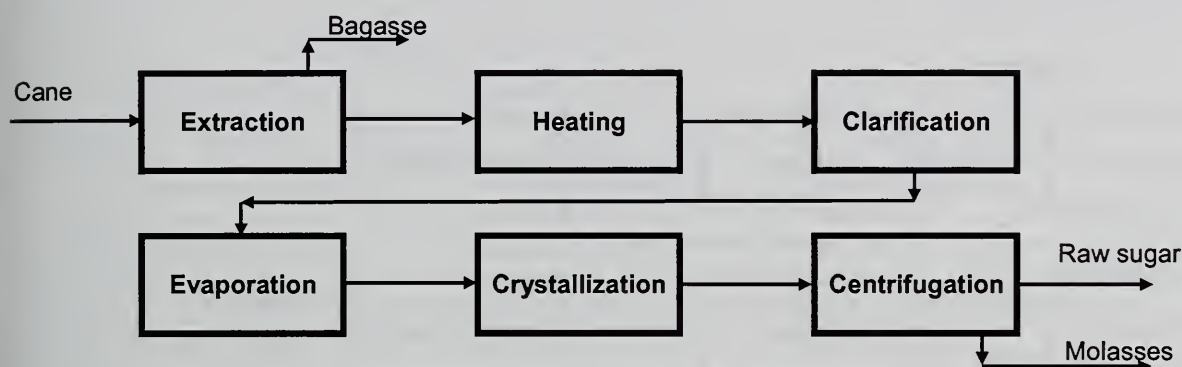

Figure 1. Raw sugar mill flow sheet,

The refining process incorporates color removal steps, filtration and crystallization. The latter is a particularly efficient purification process, but is expensive in terms of energy and equipment. There has been considerable effort made by a number of investigators to produce white sugar directly in a raw sugar mill without melting (dissolving) and re-crystallizing. The prize is the significant additional value of the product, with a white premium consistently over $60 /ton (*Todd, 1997*), as well as lower energy costs, because the mill uses sugarcane bagasse as a fuel. These new efforts have been encouraged by the developments in membrane ultrafiltration, which are incorporated in some proposed new processes.

Although great strides have been made in terms of both cost and technology, the use of membranes for decolorization of juice is limited. It is not feasible to apply membranes to raw juice, but they have been tried in many situations using clarified cane juice. This has to be done at high temperature around 90°C to ensure that no microbiological loss or fouling occurs. Steindl (*2001*) surveyed reported results which showed an average color removal from clear juice of 14% using ultrafiltration, based on different membrane molecular weight cutoff values down to 50 kDa. There is some evidence to suggest that the improvement in color of sugar may be slightly higher than the reduction in juice color implies. Nevertheless it does not appear that membranes alone can enable the direct production of white sugar. In addition, the recovery of the large amount of sugar remaining in the retentate is costly.

Membrane separation has been applied in a raw sugar mill in Hawaii but has been discontinued. No improvement in recovery of sugar results because there is only a marginal improvement in purity after membrane filtration (*Kochergin, et al., 2000*). It appears that in the future membranes may only find application in raw sugar mills in combination with other processes which add value.

It has been found that in the process of demineralizing juice using ion exchange resins, a significant degree of decolorization is achieved. This is the basis for the WSM process for direct production of white sugar (*Fechter, et al., 2001; Rossiter ,et al., 2002*). It incorporates membrane filtration ahead of ion exchange demineralization and also ion exchange decolorization using the same resins as used in a refinery, to achieve EU No 2 white sugar specifications. The process does have some other significant advantages but is not yet in commercial production. The process is represented schematically in Figure 2. Cooling of the juice is necessary before cation exchange treatment because significant inversion could otherwise occur at the low pH values experienced in cation exchange.
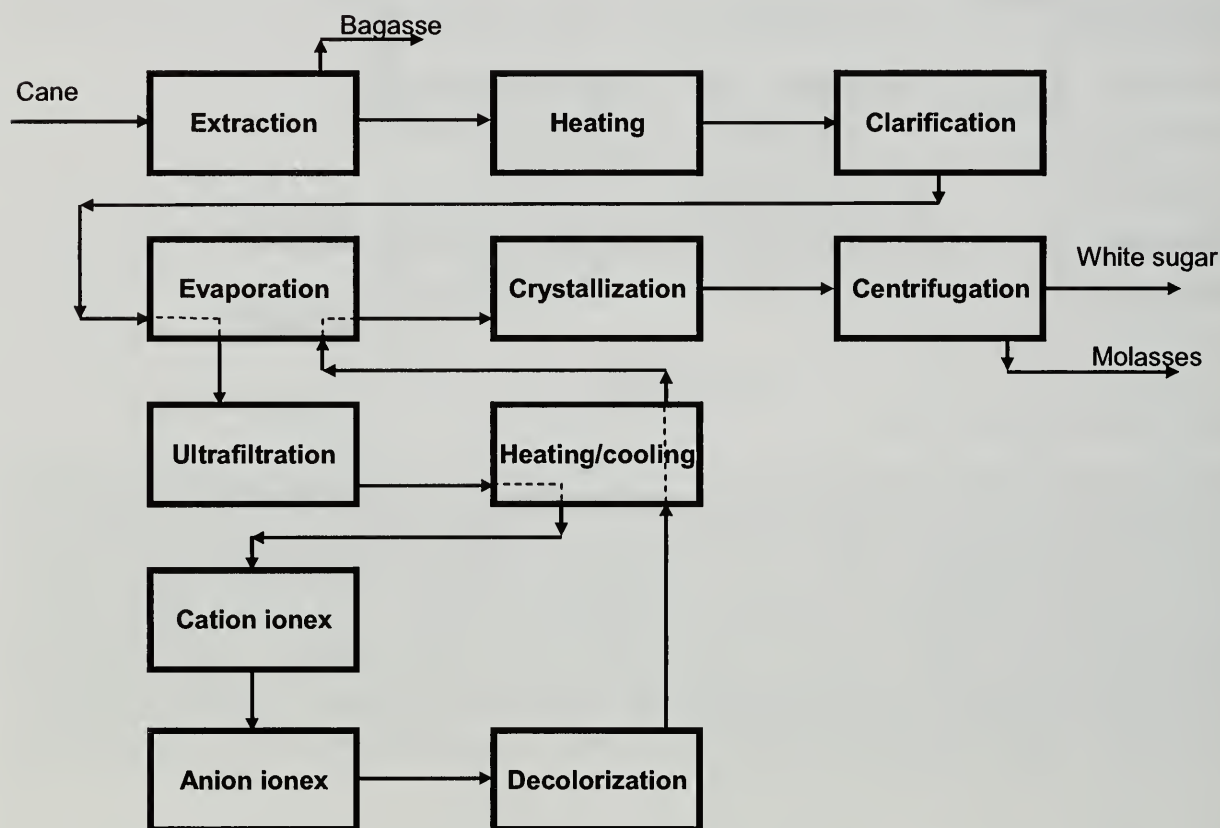
Figure 2. The WSM process for direct white sugar production incorporated in a raw sugar mill.

An alternative process removes the sugar from the juice rather than removing color and ash. This involves a chromatographic separation process, which itself has to be preceded by both membrane filtration and softening of the juice. An extensive trial in a raw sugar mill is reported by Kochergin, *et al.* (*2000*), which showed also that considerably more evaporation is required in the process.

Apart from being able to produce white sugar of EU No 2 quality, recovery of sucrose is increased in these processes as well. None of the processes proposed have, however, been commercialized.

Development of a New Direct White Sugar Production Process

The objective of the work presented in this paper was to investigate options for producing white sugar directly without the use of ultrafiltration. Mill clarified juice was treated with a combination of granular activated carbon (GAC) column decolorization and ion exchange columns to remove ash and additional color. The use of oxidants in a combination with the treatment was also investigated. A typical flow sheet illustrating the modifications to a raw sugar mill to incorporate this process is shown in Figure 3.
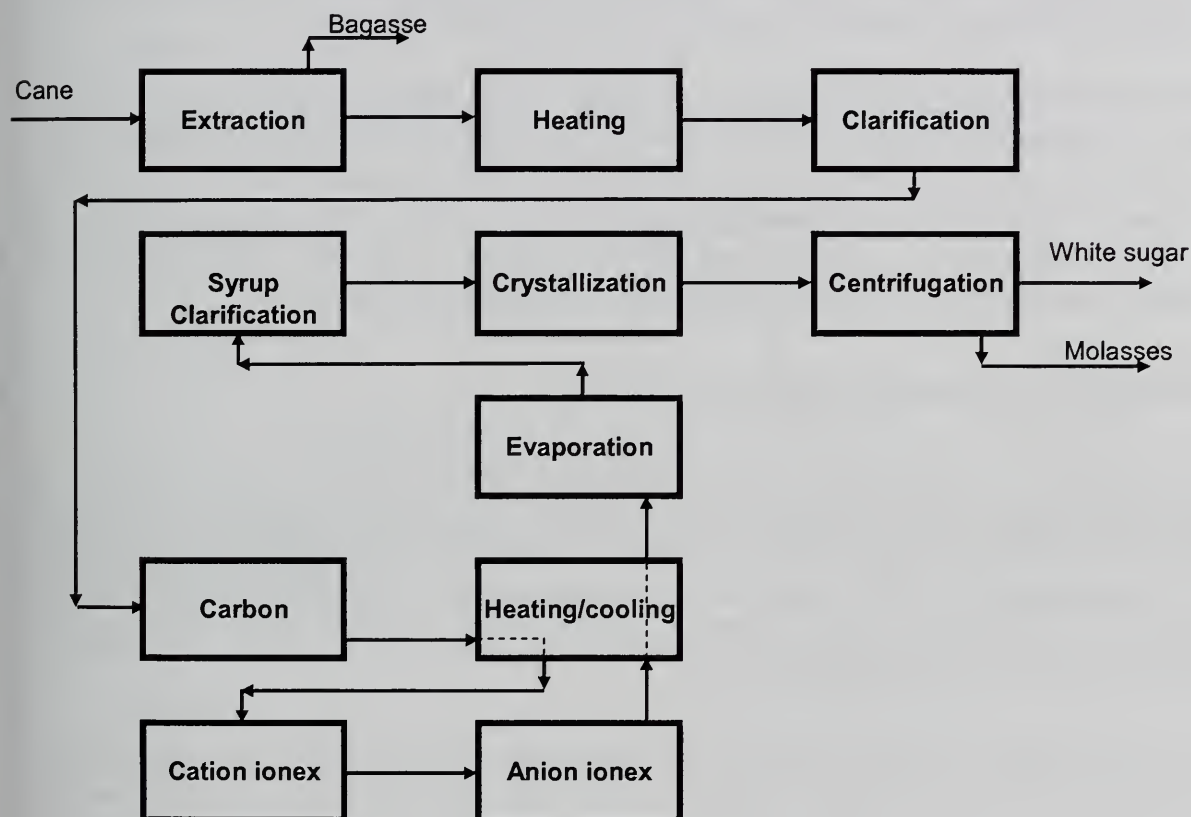
Figure 3. Alternative process for direct white sugar manufacture in a raw sugar mill.

The process investigated was expected to achieve the same advantages as the WSM process, namely (*Rossitter, et al., 2002*):
* an increase in sugar yield
* a large improvement in sugar quality
* the production of a high grade molasses for by-product use
* the removal of species that foul the evaporators
* higher heat transfer coefficients in pans and evaporators

Activated carbon is commonly used for decolorization in sugar refineries, but has not been applied in treating cane juice. Because of the low viscosity of cane sugar relative to refinery liquor, higher flow rates are expected. The carbon columns are also capable of handling some turbidity in the juice without serious fouling.

Oxidants have been used to obtain additional decolorization in refining when circumstances demand it. They destroy colorants by attacking susceptible functional groups. It appears that they do not attack sucrose and the use of ozone does not increase the production of organic acids (*Davis, et al., 1998*). These oxidants have the advantage that they have no adverse environmental effects.

Davis et al. (*1998*) report that in a refinery ozone and hydrogen peroxide both needed to be added to the melt before a precipitation step such as carbonatation. At a dosage level between 90 and 250 mg/kg DS (dissolved solids), refined sugar color was considerably reduced, averaging below 35 IU compared with an average of over 40 IU in the absence of ozone.

However, Bento (*2004*) reported that hydrogen peroxide added before resin has a number of beneficial effects. Conjugated double bonds are broken and carboxylic acids are formed. This decreases the molecular weight of the colorants and increases their propensity to be removed by ion exchange resins. Dosage rates of 500 mg/kg DS resulted in longer cycles and higher rates of color removal. As a consequence, chemical and effluent disposal costs are reduced. However care must be taken to ensure that residual peroxide does not degrade the resin.

Initial Laboratory Investigations

Pure sucrose is colorless, but may appear colored because of the inclusion of small amounts of colored material in the sugar. Color is the generic term used to cover a wide range of components which contribute to the color of sugar. Most of these compounds are complex and not easy to quantify and color is measured as the total effect of all colorants on light absorbance at 420 nm. This is not altogether satisfactory, because different colorant components behave differently in the various processing and decolorizing operations.

Work done on modeling the removal of color in the resin columns of the WSM process has been reported by Broadhurst and Rein (*2003*). Attempts were made to characterize colorants on the basis of their molecular mass, using GPC measurements. Suitable deconvolution of the retention time peaks was used to yield quantitative data on various molecular mass pseudo-components (*Broadhurst and Rein, 2003a*).

This work was done on ultrafiltered juice samples. Subsequent work was aimed at modifications to the process to obviate the use of membranes, because the cost of membrane separation militates against the economic viability of such processes, in spite of recent advances in the technology.

Granular activated carbon (GAC) was substituted as an initial juice treatment step. It is less prone to fouling than resins, and is a useful decolorizing process in its own right. A high juice flow rate through the GAC column of 15 BV/h was used. It was still necessary to cool the juice after the carbon columns to below 10°C to prevent inversion from occurring in the ion exchange columns. A comparison of the juice color obtained using the carbon column and ultrafiltration prior to ion exchange is shown in Figures 4 and 5 (*Ellis, 2004*). Juice feed color averaged about 12 000 ICUMSA units (IU). The data in Figure 5 were obtained after 10 regeneration cycles,

showing no sign of the effect of a higher juice turbidity on the performance of the resin. Regeneration of the carbon was undertaken in a laboratory furnace at 600°C.
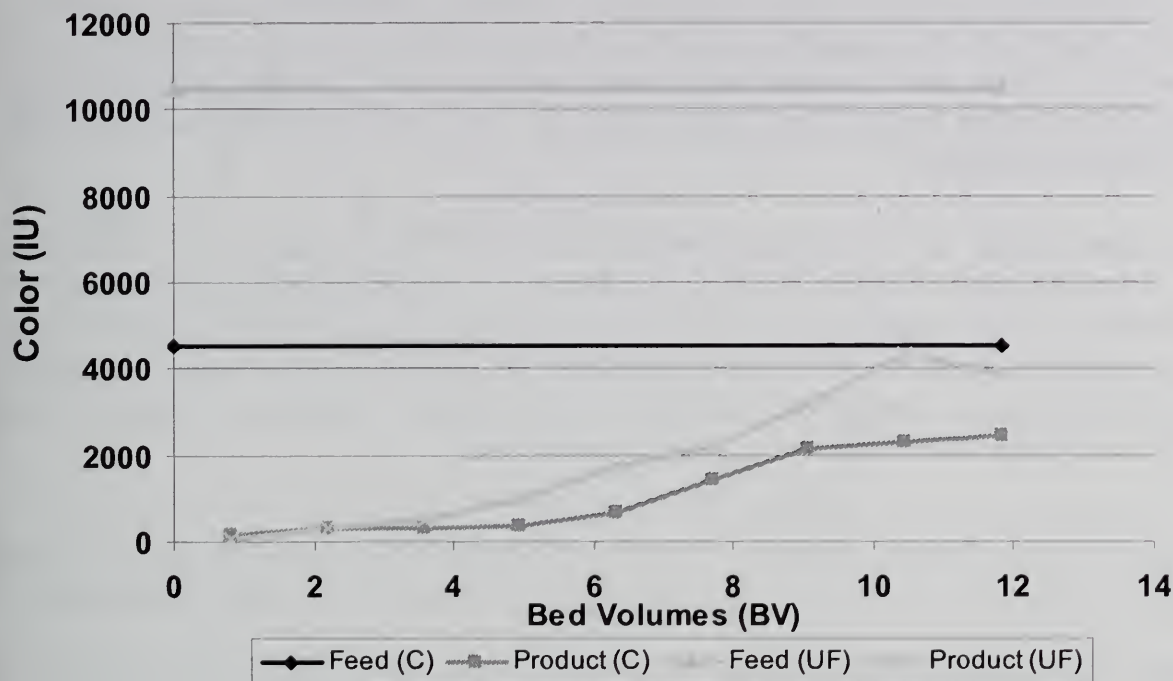
Figure 4. Comparison of juice color treated by ion exchange, pre-treatment with membranes (UF) or carbon column (C).
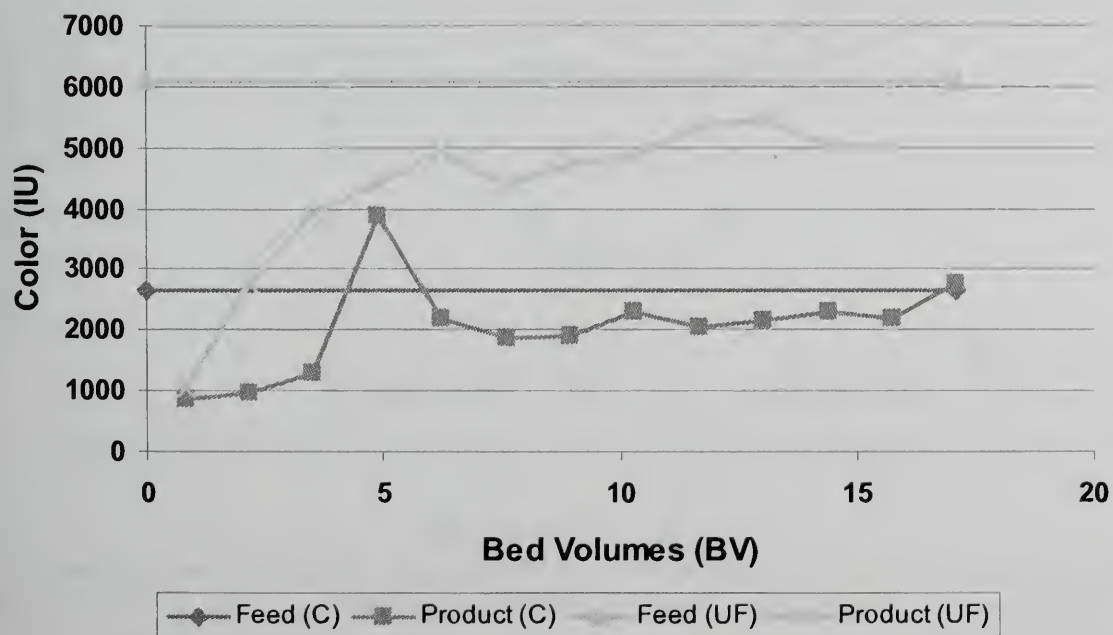
Figure 5. Comparison of juice color treated by ion exchange, pre-treatment with membranes (UF) or carbon column (C), after 10 cycles (*Ellis, 2004*).

The use of a mixed bed of cation and anion exchange resins was investigated in an attempt to obviate the low temperature operation of the resin columns, by keeping the pH in a range closer to neutral to minimize inversion.   Various ratios of cation/anion resin were tried, but performance was consistently below that achieved when running the two stages of ion exchange separately. The outlet pH was unstable and the ash removal measured by conductivity was reduced. The main effect was a substantially lower color removal. It appears that the large swings in pH obtained when the SAC and WBA resins are run in series are necessary to achieve the high color removal required.

Bento (*2004*) found that the use of hydrogen peroxide at a level of 500 mg/kg DS improved the performance of ion exchange columns in a refinery. The effect of adding hydrogen peroxide was investigated for this process in conjunction with carbon treatment. The results of some laboratory batch tests are shown in Figure 6. This indicated that additional color removal could be achieved when peroxide treatment was combined with GAC decolorization. The tests showed that little color was removed by peroxide on its own.
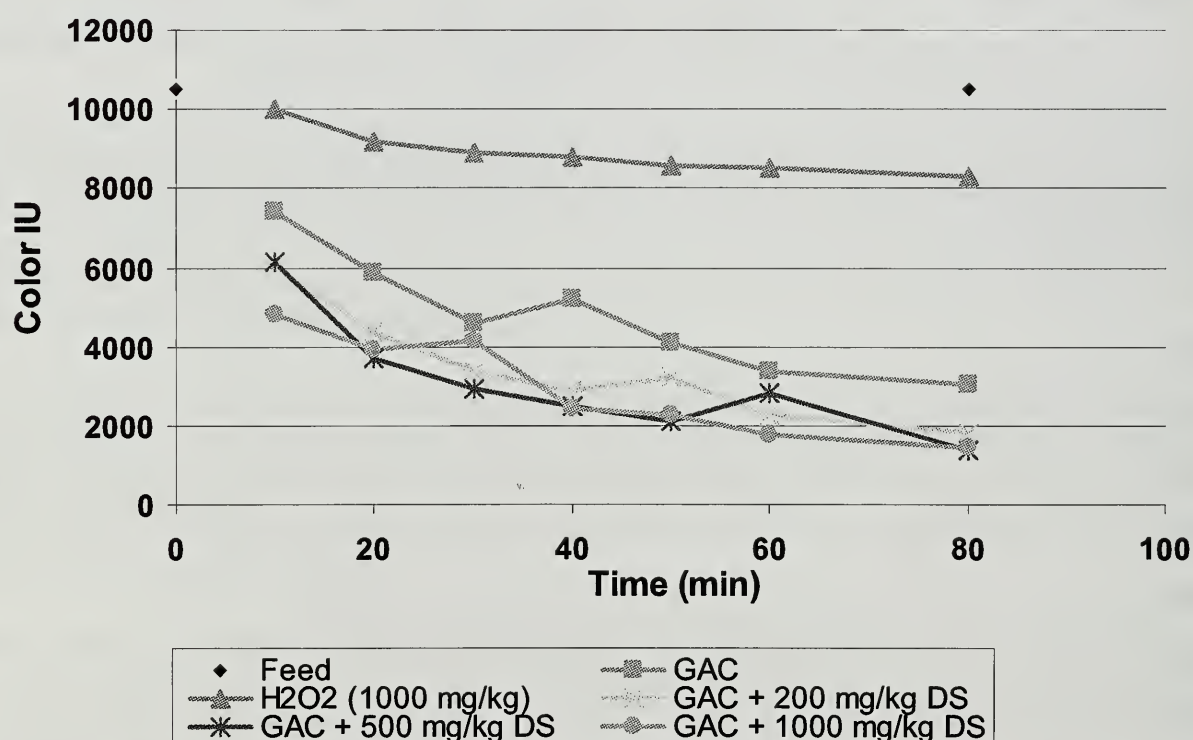
Figure 6. Batch tests on color removed from juice by combinations of carbon and peroxide treatment at 80 °C.

It was found that the modified process using a carbon column could be substantially improved by the addition of hydrogen peroxide to the juice before the carbon columns. Because the color of the juice is much higher than the color in refinery streams, a dosage rate of 1000 mg/kg DS was used ahead of the carbon columns. A reaction time of 30 minutes at a temperature of 85°C was used. The effect of the peroxide on the performance of the system is clearly evident in Figure 7 showing the color of the product, after ion exchange.
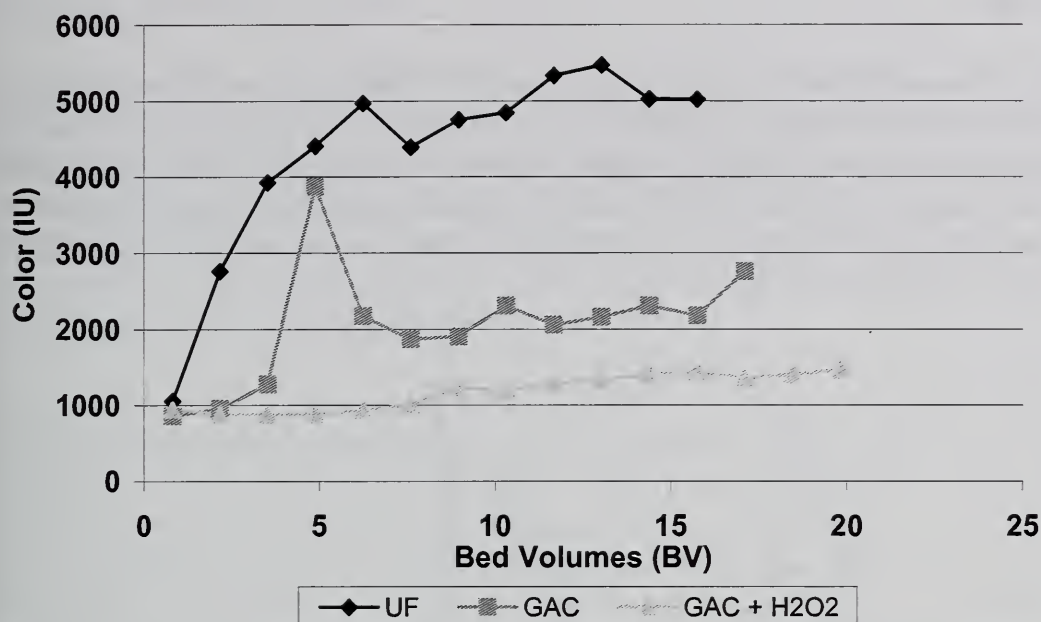
Figure 7. Final ICUMSA colors after ion exchange, comparing ultrafiltration as a first step with carbon treatment, with and without peroxide addition

Another important aspect of the resin performance is the deashing ability of the resin. Figure 8 compares the three different pretreatment methods and the treated juice final ash content in the form of conductivity. The combination of carbon and peroxide also leads to a far superior ash removal.
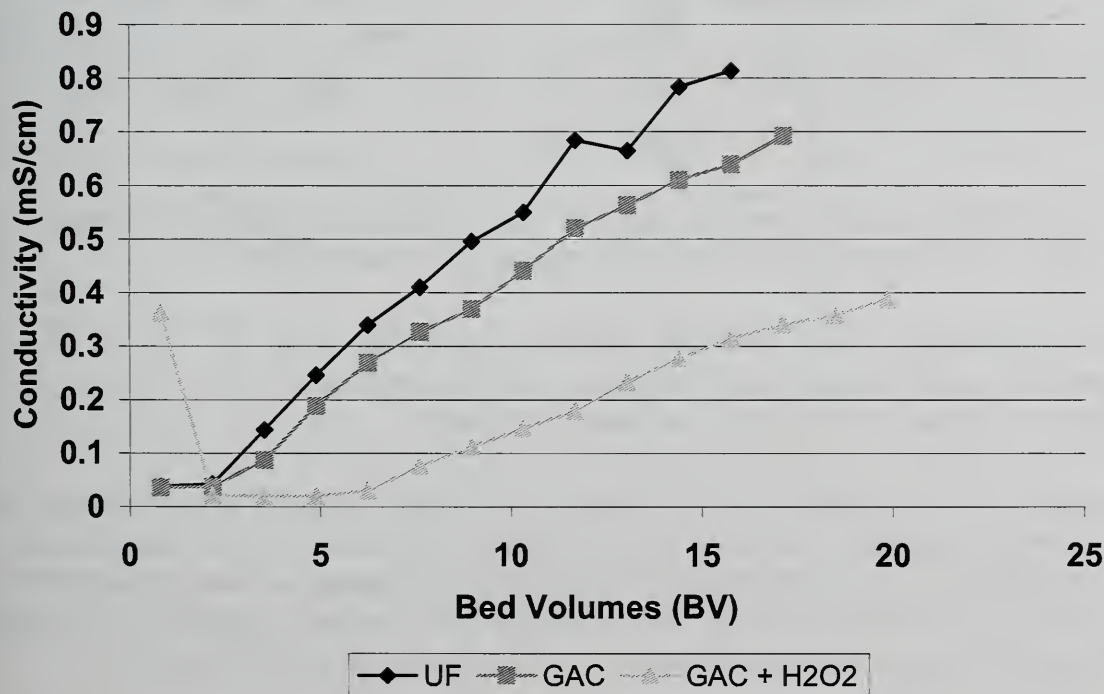
Figure 8. Final conductivity measurements after ion exchange, comparing ultrafiltration as a first step with carbon treatment, with and without peroxide addition.

First Factory Trial

A column containing 17 L carbon with a hydrogen peroxide dosing system ahead of it was installed at St. James mill in Louisiana for the 2004 season. Mill-run clarified juice was used as the feed, and the system ran continuously for 10 weeks. The carbon was chemically regenerated once/week, using a combination of washes of HCl, NaOH, ethanol and a special additive (*Bento, 2006*). Results are shown in Figure 9. On average, the juice color was reduced from 12,000 IU to about 3,000 IU.

Figure 9. Juice colors before and after treating clarified juice at St James mill for 10 weeks during the 2004 season. The vertical lines show when regeneration was undertaken.

The system proved to be fairly robust, handling periodic entrainment from the clarifier, but in spite of this still achieving substantial decolorization. The first step in regeneration involved backflow at a high rate to remove entrapped mud from the column.

Ash removal was minimal, as expected. The pH generally dropped across the column, as the acid wash appeared to take out the magnesite in the carbon which would otherwise maintain a neutral outlet pH. Variations in temperature over the test period appeared to have little effect. A more important factor was the mud carry-over from the clarifier. On two occasions after heavy rains, the column had to be back-washed with water to restore the required juice flow rate.

Second Factory Trial

A second trial was conducted at St. James mill during the 2005 season. Some changes were made to the test program. Apart from eliminating experimental problems experienced the previous year, the trial was modified to assess a different ion exchange treatment. Instead of employing strong acid cation and weak base anion columns after the carbon, as was practiced in the laboratory trials, a mixed bed of softening and decolorizing resin was employed. This mixture did not change the pH of the juice, and so dispensed with the need for cooling the juice to a low temperature to avoid inversion.

GAC was placed in two columns of 15 and 30 L capacity. The first column acted as a guard column to protect the second column from suspended solids carried over with the juice. Before the second column, juice was contacted with hydrogen peroxide. After passage through the GAC columns, juice was treated with cationic and anionic resins in a mixed bed column, to remove hardness in solution and to reduce juice color. The installation worked continuously for two months with clarified juice at a flow rate of 1 BV/h (on total carbon). Average data are shown in Table 1. A reduction of 81.5 % in juice color was obtained over the test period (75.6 % on carbon and 24.1 % on resin).

Table 1. Composition of clarified juice before and after treatment.

	Brix	Color (IU)	Turbidity (NTU)	pH	Ash (g/100 g DS)
Clarified juice	14.3	9,070	212	7.7	3.5
Juice after 1st GAC column	14.3	6,870	191	5.9	3.7
Juice after 2nd GAC column	14.1	2,220	187	5.6	3.9
Juice after ion exchange	13.9	1,680	165	6.0	4.0
Reduction (%)		81.5	22.2		

The variation in color measurements seen through the test period is shown in Figure 10 and the decolorization in each cycle in Figure 11. It is apparent that the extent of the decolorization reduced over the trial. This is largely due to the performance of the first GAC column, which was handling juice not treated with peroxide and, in addition, was regenerated with spent regenerant used in the second GAC column. After cycle 8 (8 weeks of operation), roughly one-third of the carbon was replaced with fresh carbon and decolorization at least in the second GAC column showed improvement.
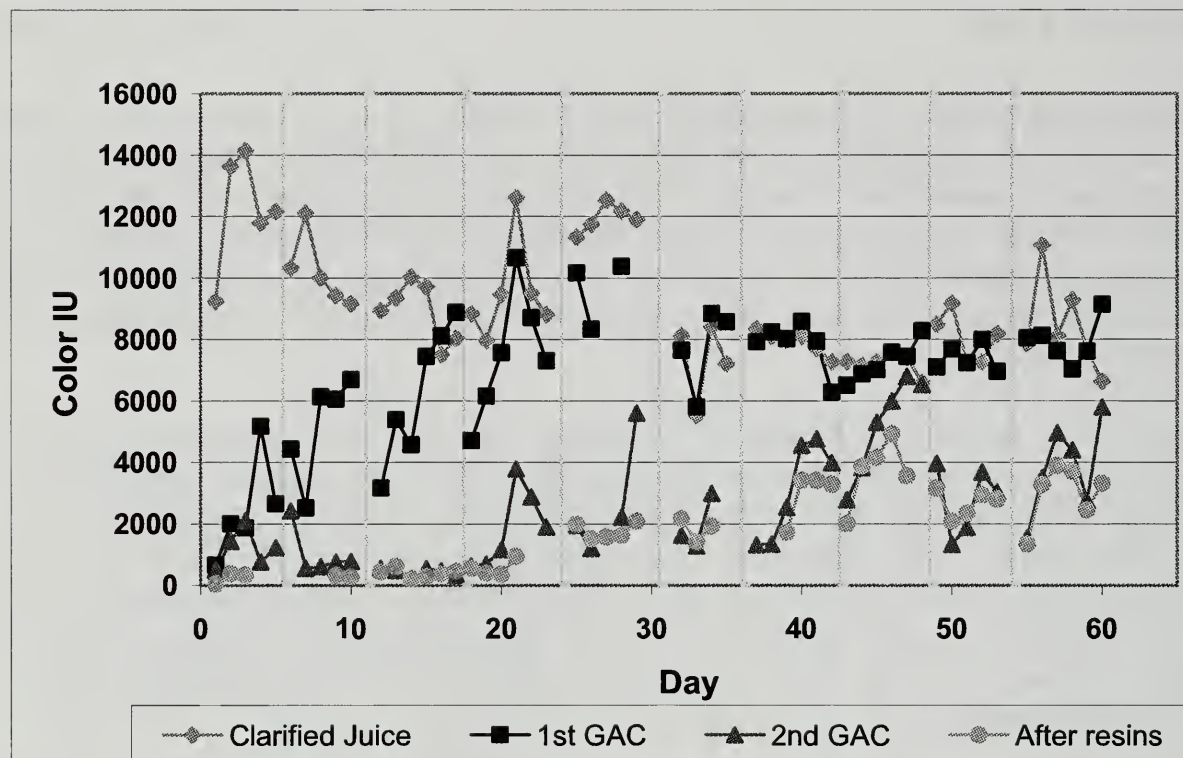
Figure 10. Measurements of color in juice streams during trials (the vertical lines represent regeneration times).
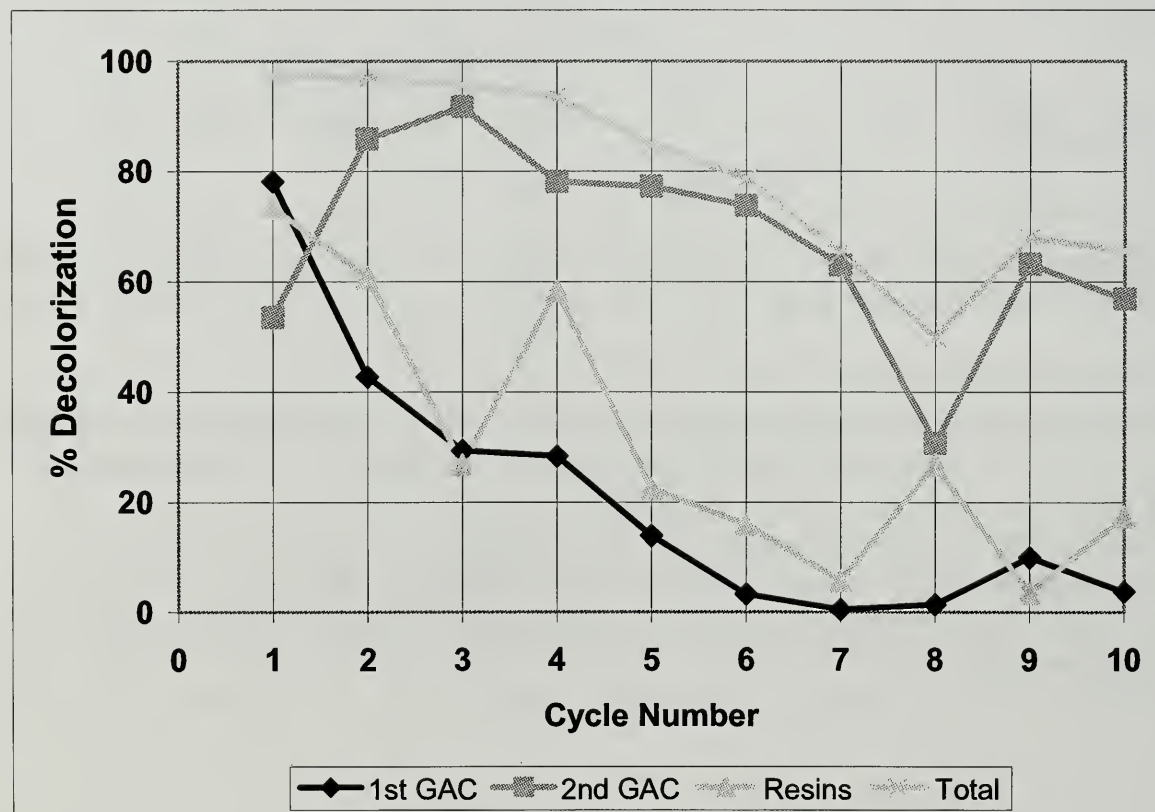
Figure 11. Decolorization achieved in 2005 trials.

A turbidity decrease of 22.2 % (Table 1) and hardness (calcium and magnesium) reduction of 88.5 % were observed (Table 2). An increase in lactic acid through the columns was also registered at times, because it was not possible to keep the temperatures consistently above 80 °C in the pilot plant.

Table 2. Composition of juice.

	Apparent purity (%)	Ca + Mg content (mg/kg DS)
Clarified juice	87.4	4240
Juice after 1st GAC column	88.6	4460
Juice after 2nd GAC column	88.2	4350
Juice after resin column	89.0	489
Reduction		88.5 %

Resin cycles varied between three and four days. After each cycle, the resins were washed with condensate water followed by regeneration with NaCl and NaOH. The performance of the ion exchange resins was affected by contamination of the "condensate" stream with low quality water, which adversely affected regeneration. Nonetheless it appears that this option will not reduce the ash content of juice sufficiently to be able to achieve EU No 2 sugar ash specifications; an ash reduction of at least 75% is necessary.

After each five day cycle, carbon was washed and regenerated chemically. Using a new regeneration process developed at Audubon Sugar Institute, a special solvent regeneration was applied, using ethanol, caustic soda and a special additive (*Bento, 2006*). Color loading was high, well over 200 t DS·IU/L resin per cycle.

Some analyses done on four samples during one day of the trial gave the values of color and Indicator Value shown in Figure 12. In this case the Indicator Value is the ratio of color measurements at 8 and 5 pH instead of the usual values of 9 and 4 pH. This shows a steady decrease in Indicator Value, suggesting that a substantial part of the natural colorants (phenolics and flavanoids) is removed. Bento (*2006*) evaluated the propensity of other juice colorant types to be adsorbed on GAC.

Figure 12. Measurement of color and Indicator Value.

Laboratory Optimization Trials

Optimization of the process involves studying the GAC process in terms of its capacity in relation to operating conditions, particularly flow rate, temperature and peroxide dosing rate and the effect on the subsequent downstream ion exchange step. The frequency and cost of regeneration is also important. Laboratory work has been aimed at obtaining equilibrium data, generating isotherms under different conditions, and kinetic data based on mass transfer in packed columns.

The capacity of the ion exchange columns is largely determined by the reduction in ash required to meet the required sugar specifications. There is more flexibility in choosing the operating conditions for the GAC columns. The approach to optimization has involved a number of laboratory trials under varying conditions, and applying a mathematical model of the type used by Morley (*1988*) and Broadhurst and Rein (*2003*). This is a linear driving force model, assuming axial dispersion has a negligible effect.

The mass transfer coefficients are substantially affected by the flow rate, favoring high rates through the GAC columns. This results in smaller columns but more frequent regeneration.

Application to a Raw Sugar Mill

The flow sheet for a raw sugar mill using this process is shown in Figure 3. A syrup clarifier is incorporated to ensure that the suspended solids content is reduced sufficiently for the high quality sugar required. It goes without saying that juice clarification must be well-controlled to ensure that the columns are not subject to carry-over from the juice clarifiers at any time.

A number of options exist for the configuration of the GAC system. Instead of a guard column, a pulsed bed GAC system would on a regular basis discharge the carbon layer which could get fouled by carry-over from the clarifiers. A substantial advantage of the chemical regeneration system however is the ability to configure the GAC system in the form of a simulated moving bed. The advantages, as with ion exchange, could be substantial (*Hubbard and Dalgleish, 1996*), in particular reducing the cost of regeneration and reducing the amount of sweet water produced.

The use of chemical regeneration of GAC seems viable, at least over a period of 10 weeks. Beyond this time it may be necessary to introduce thermal regeneration of part of the GAC to keep the process operating efficiently. Transport of carbon to an outside facility would be the best option if transport costs allow it. In thermal regeneration, something between 4 % - 10 % of the GAC is lost in the regeneration process. Thus after 10 regenerations a significant amount has already been lost in the process of thermal regeneration. This is avoided in chemical regeneration.

The use of ethanol in regenerating GAC is particularly suitable in a sugar mill which also produces ethanol. The spent regenerant can be sent back to the distillation plant for a total recovery of the ethanol. Broadhurst and Rein (*2003*) also showed that a periodic ethanol wash also improves the decolorization capacity of the SAC column, and this could be considered in a sugar/ethanol production facility. A major advantage might also be the elimination of the need for refrigeration before ion exchange, if inversion to reducing sugars is not important in the context of a mill producing ethanol.

The cost of regenerants for the process presented here is estimated to be roughly $0.50 for a color loading of 1 t DS·IU/L of carbon per cycle. In the Louisiana context, this translates to 0.20 US cents/lb sugar for regenerating GAC. The cost of regenerating the ion exchange columns is 0.76 US cents/lb sugar, giving a total of about 1 cent/lb. This can be reduced to 0.67 cents with some reuse of the HCl and NaOH. The disposal of effluent is a problem or perhaps an opportunity depending on the particular mill's circumstances. The inorganic material removed is that present in the cane, and is particularly high in potassium, which has considerable fertilizer value. Regeneration of the WBA columns with ammonium hydroxide can significantly boost the fertilizer value of this regenerant (*Rossiter, et al., 2002*). The use of the regenerant as a fertilizer could be the best solution if it could be integrated into the combined operation of growing and milling without any detrimental effects on the environment.

Additional power and steam requirements are expected to be modest. Because of the virtual absence of scaling expected in all evaporator vessels, no additional evaporator capacity will be necessary, but minor changes might be expected to achieve the desired steam balance.

Conclusions

The potential for low cost white sugar production is excellent. Combined with the advantages of higher sugar recovery, better molasses quality and the elimination of evaporator scaling, prospects for direct white sugar production are good. Further trials to establish optimal design criteria are on-going.

Acknowledgements

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Savings in Processing Aids:
Developments within AGRANA/Zuckerforschung Tulln

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Introduction

It was not too long ago that the production costs of white sugar were financed by sales of by-products like molasses and dried pulp. Only the beets needed to be paid for by the revenues generated by white sugar. The energy crises in Europe changed this situation for the very first time. In the mid-1990s companies started to take into account the costs caused by the application of processing aids. This also gave rise to new fields of research and development as well as process optimization.

Among these new projects were measures for the implementation of anti-foaming agents (or antifoams) and the development of an apparatus for objective evaluation of the demand for anti-foaming agents in the extraction area. Furthermore a quite simple but very robust device for determination of residuals of calcium content for optimal dosage of alkalising medium or scale inhibitors was developed. Finally a program for optimal dosage of milk of lime in the juice purification was developed, which led to savings of limestone and coke.

All these developments derive from some kind of "historical" evolution. The generated devices incorporate similar construction principles and control mechanisms. In every case the constructions were planned to be easily maintained and robust. At this very beginning it is also worth mentioning that the reduction in processing aids is not only a technical issue but also a psychological one, which will be explained in more detail later on.
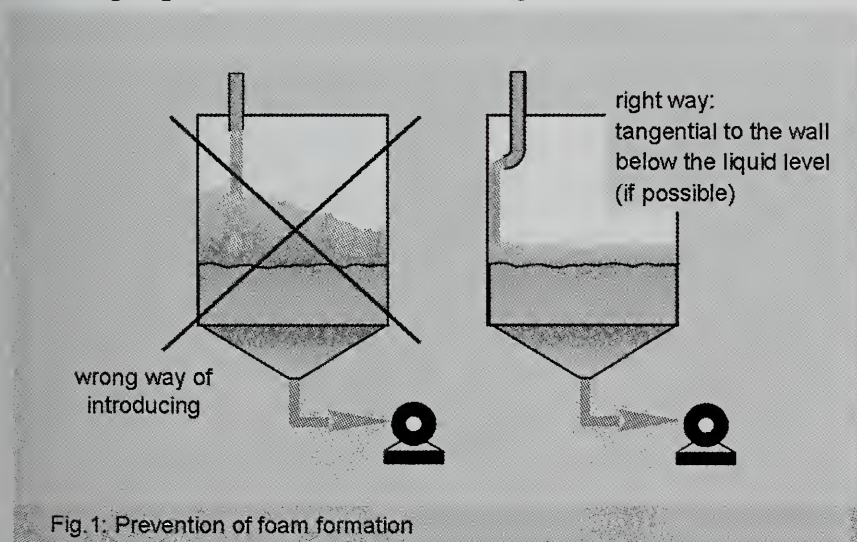
Savings in Anti-Foaming Agents

In 1994 we were asked to investigate the circumstances under which one of the Austrian sugar factories had high consumptions of anti-foaming agents.

Problems caused by the formation of foam frequently occur within the field of food technology. Concerning sugar technology, problems with foam formation have an impact on almost every processing step, mainly within the extraction area and during juice purification. Problems with foam formation are also sometimes reported at evaporation and crystallisation steps. Among other substances, saponins (surface active substances, located in the outer layers of the beet) play an important role in the formation of a stabilised two-phase-system containing gas and liquid. The problems caused by these substances are ebullition of tanks connected with sugar losses as well as abnormal activity of pumps. In general, measurement and control operations are highly sensitive to the presence of surface active substances.

In technical processes it is very usual to prevent the formation of foam by applying various surface active substances (anti-foaming). Another possible method is the destruction of already existing foam (defoaming). Under special circumstances financial costs of these products represent an important part of all costs of processing aids.

Despite all inventions for the chemical or mechanical destruction of foam, the prevention of its formation is still the best way to deal with it. Figure 1 displays false introduction of a potentially foaming liquid into a tank and the right method.

right way:
tangential to the wall
below the liquid level
(if possible)

wrong way of
introducing

Fig. 1: Prevention of foam formation

With convenient construction of tanks and correct positioning of pipe work for the liquid inlet into a tank (tangential to the tank wall, below the liquid level) many problems can be avoided.

If these measures do not turn out to be sufficient, there is still another option despite the application of surface active chemicals: mechanical destruction of foam.
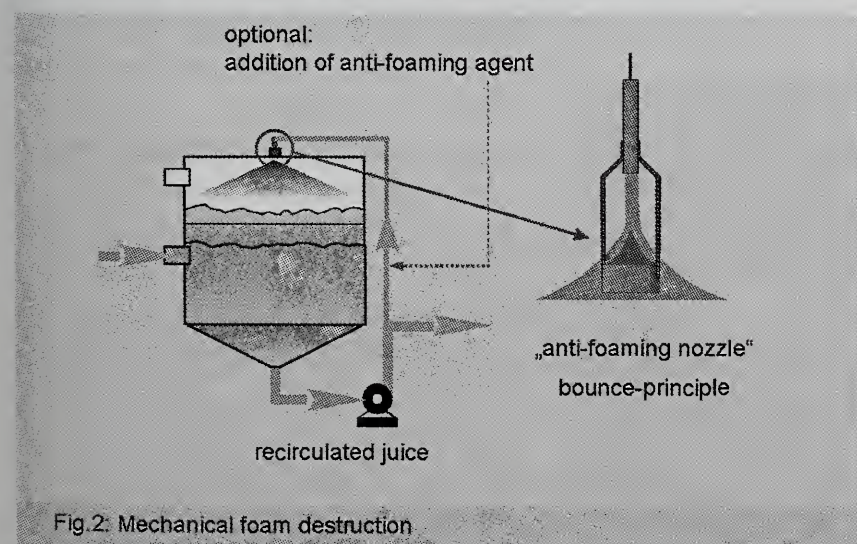
Figure 2 displays an example for mechanical foam destruction by splashing juice through nozzles on its own liquid surface.

This variant for destroying foam operates by splashing juice through nozzles onto the foaming surface of the liquid. For reasons of energy savings, the liquid used in the process should not be additionally added water. At best, foam destruction is carried out with juice which is available at

optional:
addition of anti-foaming agent

"anti-foaming nozzle"
bounce-principle

recirculated juice

Fig.2: Mechanical foam destruction
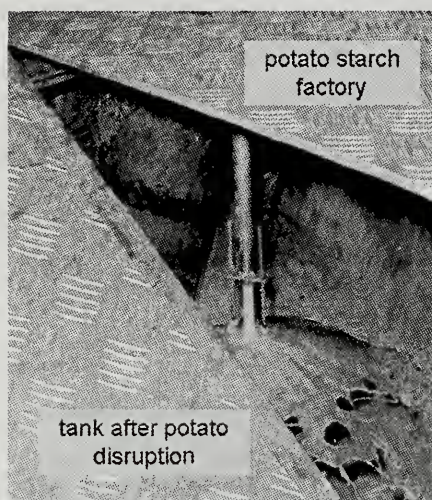
Fig.3: Mechanical foam destruction

this certain stage in the process. A small recirculated component current of the juice is splashed from a short distance over the surface. Still, there is one quite frequent problem with this method which demands for specially designed nozzles: used liquids may contain small solid parts. Figure 2 displays this kind of nozzle which operates by applying the bounce-principle and does not feature any narrow spaces. This special construction prevents the nozzle being blocked by small solid particles. For juices that contain sand, a fitting and robust material needs to be chosen. Figure 3 displays the impact on foam of such kind of nozzle as it is applied in starch factories.

If mechanical measures cannot be established or do not lead to the desired results, anti-foaming agents have to be applied. Anti-foaming agents have to be used at very low and absolutely necessary concentrations and are recommended as the very last alternative for foam destruction.

One step towards this direction is the application of so-called foam detectors (Figure 4).

These detectors are made of two metal electrodes or probes which are completely isolated with exception of their bare tips. In case of foam formation a connection between the electrodes is established and the generated signal is used for triggering off a dosage of anti-foaming agent. Permanent linkage between the two electrodes after the dosing procedure is one potential source of error. Integrated flushing devices are rather simple but very effective constructions to deal with this problem. By periodic spraying of condensate (flushing device) linkages deriving from foam formation disintegrate and the probes work accurately. In the meantime all foam detectors at Agrana factories are operating with integrated flushing devices (1).

Fig.4: Foam detector

Fig.5: AFO – Antifoam Optimizer

The so-called Antifoam Optimizer (AFO) is a more complex apparatus for optimised application of anti-foaming agents (Figure 5). This special apparatus is operating in a separate current and optimises the addition of anti-foaming agents. The system consists of an overflow tank which is flushed and then filled up with juice (tower juice, press water). Then air is injected into

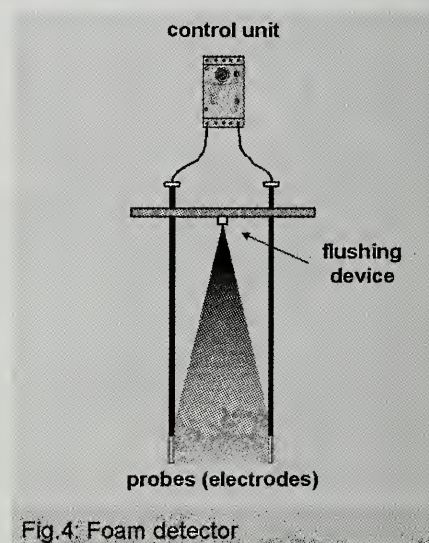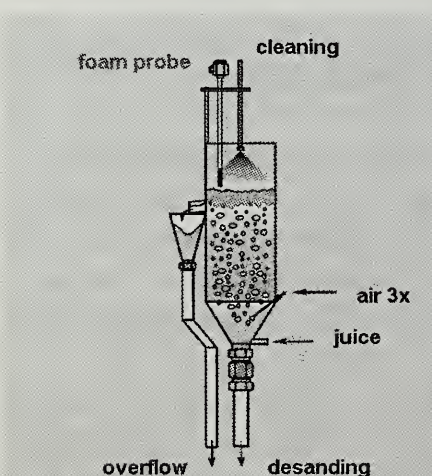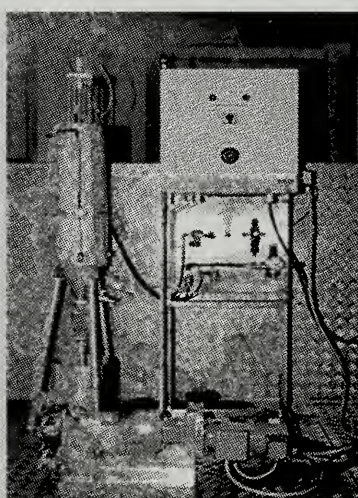the juice over a special frit and the foam formation observed. If the foam formation reaches the electrodes within a certain time span (4 min) the system raises the dosage of anti-foaming agent. For the inverse case, the dosage amount is reduced. Due to safety reasons a reduction represents only one-third of an increase. Depending on the tendency of the juice to foam the accurate amount of anti-foaming agent is added. Supervision of the system and the dosage of anti foaming agents are managed either over local control panels or over a process control system. For proper functioning of the apparatus some special knowledge and experience is needed concerning error-free juice supply and positioning of the frit and the electrodes. Figure 5 displays also an image of the whole construction.

The system offers the advantage of dosage regulations in both directions. As already highlighted at the beginning, psychology plays an important part in all these optimisation measures. A major part of responsibility about the right dosage times and dosage amount is transferred from the operating personnel to the equipment and therefore put on a more objective basis. Also ongoing rivalry and competition between personnel of factories and shifts within factories for achieving the lowest consumption in processing aids is an important factor.

The next image (Figure 6) proves that all this is not just pure theory. The figure displays the progression of anti-foaming agent consumption for the Tulln factory and AGRANA as a whole.
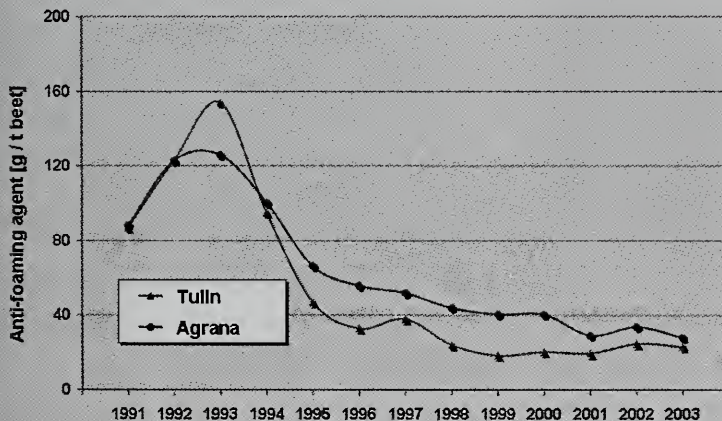
Fig. 6: Progression of consumption of anti-foaming agents within Agrana

Compared to very high consumption figures in 1992 and 1993, a reduction of more than 75 % was achieved. Moreover the average consumption of the early nineties was reduced by 50 %.

The values displayed in the figure are calculated in ppm, which is permissible since practically all the time the same product was used. Concerning different products with variable prices and effective concentrations, cost estimations would be the deciding factor.

The AFO-system is also very suitable for testing various products on their efficacy.

Optimal Dosing of Alkalising Medium

Depending on the beet quality (little content of alkali ions, high content of α-amino nitrogen, high invert sugar content) and the process settings (intentional infections) alkalising medium has to be added during juice purification in order to keep the residual content of calcium in thin juice low. It is highly important to apply the optimal amount since too little dosage leads to high content of calcium ions in thin juice with a well known impact on the following steps of the process (scaling in the evaporator station). Overdosing, on the other hand, results in high pH-values in thin and thick juice and causes troubles during crystallisation. The problem is even

harder to control with steadily changing beet quality. Moreover the detection of residual hardness in juices after purification is an important pre-condition for optimal dosage of alkalising medium.

The next development describes a simple and robust detection method suitable for analysing residual contents of calcium in juices. During investigations on foam destruction by chemical means, an old calcium determination method was rediscovered and automated by combining it with a foam probe. It utilises the effect that soap solutions precipitate to calcium-salts and therefore do not generate foam.

This method is known as the "Clark Method" for the determination of hardness in water which was mentioned first in the literature in 1852. The now automated method operates with an apparatus which includes the same components as used for the determination of foam formation in juices (2). The construction is rather simple but is very robust (Figure 7).
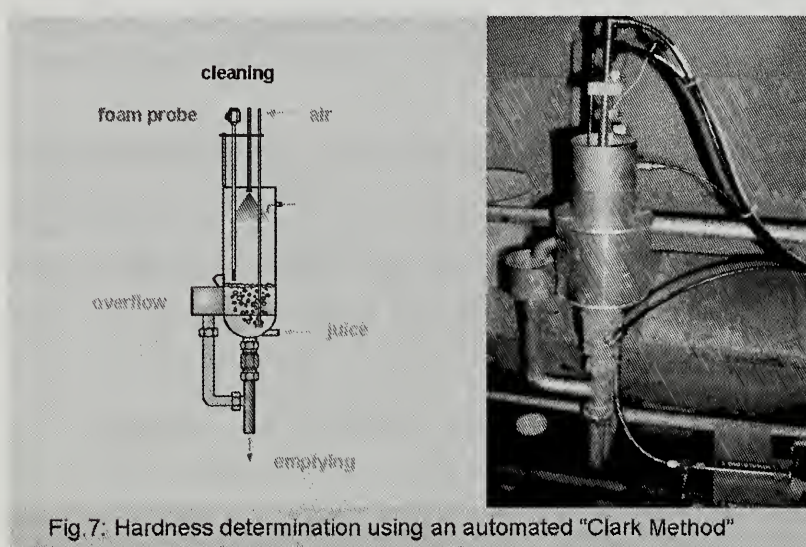
Fig.7: Hardness determination using an automated "Clark Method"

In this procedure small dosages of soap solution are added to a definite amount of juice in which air is injected over a frit. The soap forms insoluble salts with Ca and Mg ions so that no foam formation occurs in the solution. The slightest surplus of soap solution causes foam formation. Foam formation then is detected by one electrode analogue to the AFO described before. By knowledge of the added amount of soap solution the initial concentration of Ca and Mg in the juices can be calculated. After completion of the titration the juice is discharged through valves and the tank as well as the electrodes are flushed.

Compared to the AFO the construction is a little bit smaller since almost clear juices are processed and the pipelines have smaller diameters. Less juice volumes lead to lower consumption in titration medium. This is advantageous with respect to supply logistics. The operating of this little device, which we named LISA (Lime Salts Analyzer), needs special knowledge for optimal operation and useful data outcome. Fields of application cover the optimal dosage of alkalising medium, supervision and control of ion exchangers for softening as well as dosage of scale inhibitors. The advantages of the device are its robust construction and low maintenance effort. Moreover an unproblematic titration medium (palmitic acid solution, also available in kosher) is used. This device is used not only by Agrana but also by other European companies.

Savings in Limestone and Coke Consumption

Concerning juice purification an old rule of thumb said that about 100% CaO calculated on nonsugars in raw juice is necessary for processing. This amount corresponds to limestone consumption figures of 3.0 to 3.5% on beet. For burning of limestone normally an amount of 7 to 8 % of coke is needed. These figures indicate that the consumption of limestone and coke as well are highly cost intensive factors in sugar production and reveal great potential for financial savings. Limiting factors in the reduction of lime are the quality of juices concerning thermo-stability, residual hardness and colour as well as filterability properties.

First investigations took place during the 1999 campaign in the Tulln factory. This factory operates with a filter station (candle filters after 2nd carbonation). A clear relationship was found between measured pressure in the filter station and consumption of milk of lime.

With this relationship we created a command variable deriving from the quotient of filter juice volume and filter pressure (m^3 juice per bar filter pressure) which we simply referred to as "specific filter performance."

Based on this command variable a computer program was designed which we named LIMOS = Lime Optimization System (3).

This program operated by taking over data for the filter pressure and filter juice volume from the process control system, calculating the specific filter performance and comparing this with the set value. During a test stage the program output was a dosage suggestion depending on the outcome of the comparison. Later on a direct signal triggered dosage of milk of lime. The program also includes settings for lower limit values.

In this phase of development LIMOS was not designed as a special stand alone device for certain measurements like AFO or LISA - it was just a computer program (Figure 8). Regulation operations of the program are done in the same way like operating staff should do, but more consequently, especially when dosing of milk of lime has to be reduced.
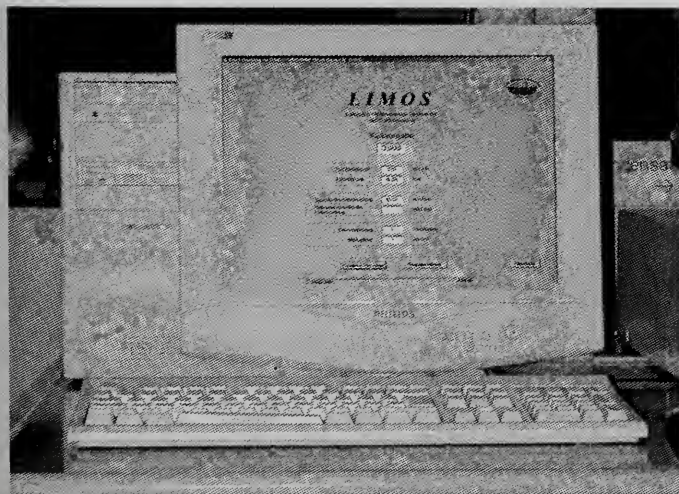
Fig.8: LIMOS – Lime Optimization System

The picture in Figure 9 illustrates the mode of operation of the program (4). It displays the two parameters "specific filter performance" and "dosed amount of milk of lime" over the period of 2 days of campaign. If the filter performance displays values above or below a defined range (e.g. 9.6-10.2) the dosing of milk of lime is either raised or lowered depending on the current deviation. This was the case for several times as
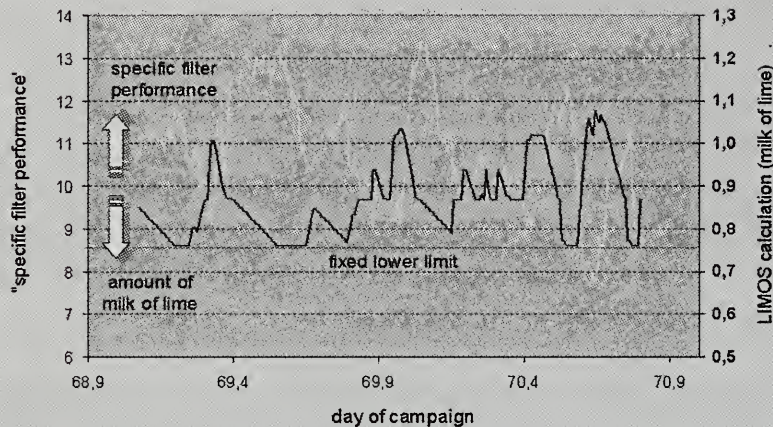
Fig.9: Mode of operation of LIMOS (2 days)

displayed in the figure. Due to safety reasons, the program operates with a minimum limit value and even at times with very good filter performance the measured values do not fall below this minimum limit (also indicated in Figure 9). There is a similar limit for higher values which were not reached in the displayed time span.

Figure 10 displays a longer period of the campaign. The program triggered off dosages of milk of lime over the whole period. Due to good beet quality (and therefore good filter performance) in the second half of the displayed time span, the dosages of milk of lime were below a so-called psychological limit which the operating personnel probably would not have undershot. The running of the program for optimisation of necessary dosage amounts of milk of lime depending on the current situation lead to significant savings of milk of lime throughout the campaign.

These savings are displayed in Figure 11. The program performed 11,990 calculations over the whole campaign. Because of these frequent calculations, very small amounts of milk of lime were used, especially in the middle of the campaign. Over the whole campaign a total consumption of 2.02% limestone on beet was necessary.
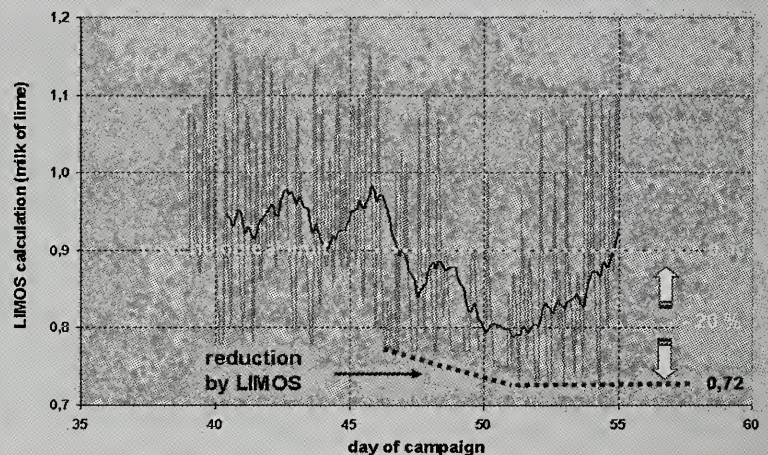
Fig.10: Mode of operation of LIMOS (25 days)

This figure covers the gross limestone consumption including splitter and unburned, also including those amounts which were needed for alkalinisation of flume water, which means it does not just simply refer to juice purification; this was during the 2002 campaign in the Tulln factory.
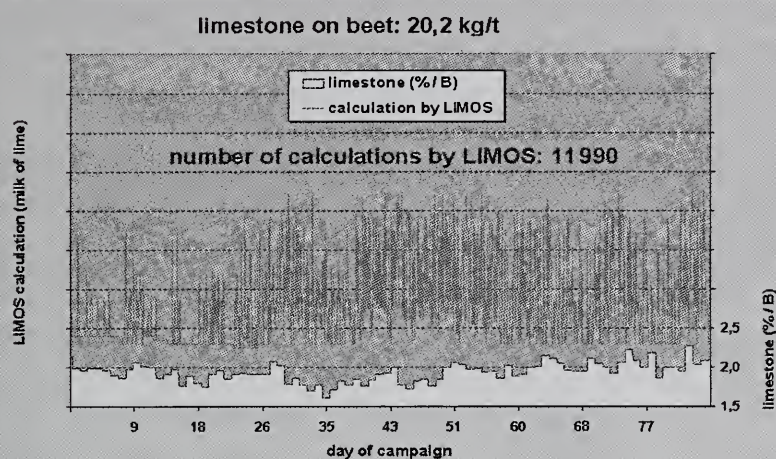
Fig.11: Mode of operation of LIMOS (whole campaign)
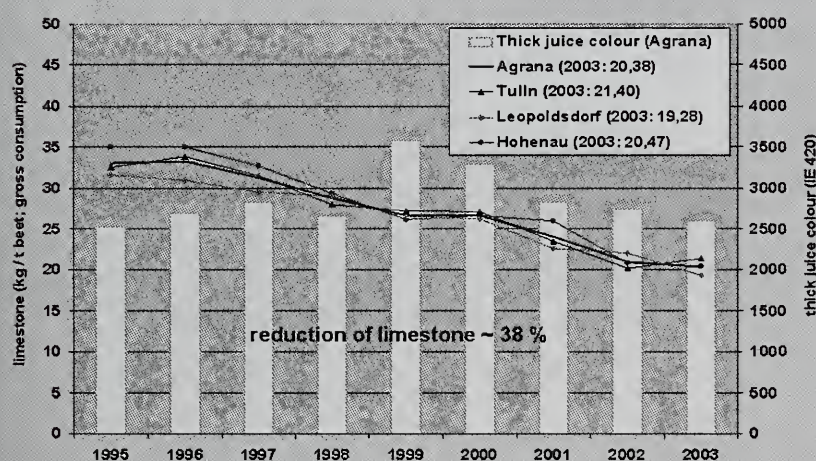
Fig.12: Progression of limestone consumption (1995 – 2003)

Figure 12 displays the development of limestone consumption in Austria over the last few years. Consumption figures for 3 single factories plus mean values for AGRANA as a whole are illustrated (5). Mean values for AGRANA concerning limestone consumption decreased from 3% to approximately 2% over the displayed time span. In addition to figures for limestone consumption in kg/t beet, colour values for thick juice are included. Alongside with all efforts for savings in lime it is highly important not to worsen quality properties.

This compromise is well demonstrated. From the given picture it is not valid to derive a causal connection between better thick juice colours and lower consumption of limestone. This has to do with shorter campaigns and changes concerning delivery logistics of beets.

However, the described program was only applicable in factories with filter stations. During the reconstruction of the juice purification in the Tulln factory, the filter station was replaced by two clarifiers. Due to these changes a new command variable was needed. First of all, the membrane filter press which is used for water removal of carbonation slurry concentrate was thought to be fitting. But it has a delayed signal which is not very characteristic either. A mini-clarifier which had already proved to be very versatile and had delivered good results in other projects performed very well indeed, but the operating expenses were much too high. Finally it was decided to build a mini-filter for obtaining the command variable. This was done by reverse engineering of a candle filter using its original but shortened candle. This construction provided a very robust signal with passable operating expenses (Figure 13).
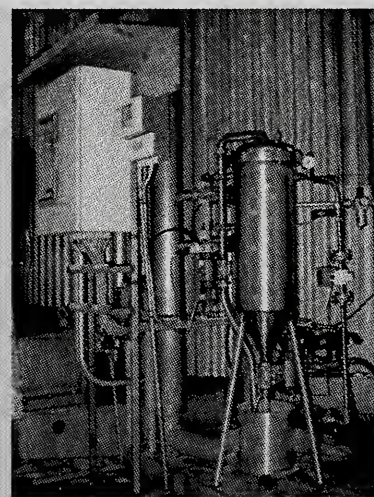
Fig.13: Mini thickening filter

The apparatus had its own sequence control which was conducted similarly to the full scale (technical) device. That also included correspondent cleaning cycles (3% formic acid; once in 24 hours). The filter is replaced once a week and maintained by external cleaning procedures (6).

The average pressure within an adjustable time interval (6-8 min) serves as a command variable. Pressure measurements are performed every 2 seconds. The signal is transferred to LIMOS which then forwards its own signal for regulation of milk of lime dosages to the process control system. LIMOS had to be adjusted and re-programmed for correct operation under the new conditions. After some test runs during the 2004 campaign, the system operated in 2005 practically over the whole campaign in the sugar factory in Tulln.
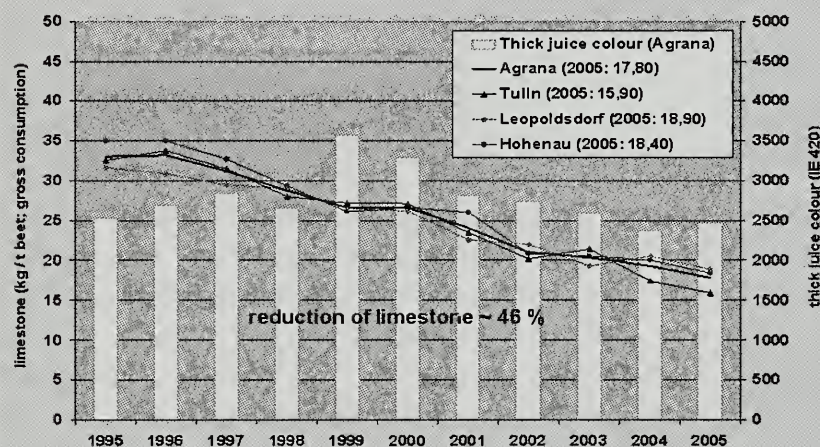
Fig.14 Progression of limestone consumption (1995 – 2005)

Figure 14 displays results of the last two campaigns. The downward tendency in limestone consumption continued without any noteworthy change for the worse concerning thick juice colours. The factory in Tulln had a gross consumption of limestone of 15.9 kg/t beet; the AGRANA average was 17.8 kg/t beet (7).

Summary

Measures for savings in processing aids within AGRANA Austria:

Savings in the field of anti-foaming agents were achieved by reconstruction measures, mechanical foam destruction, and application of foam probes with integrated flushing system and finally development of an apparatus operating in the bypass for objective determination of necessary amounts of anti-foaming agents.

The consumption of alkalising medium was reduced by application of an automatically operating system named LISA. The determination of hardness in this system is based on the "Clarke Method". It is also used for the optimisation of scale inhibitor dosages and softeners based on ion exchangers.

The third section of the presentation highlights an application which offers the highest potential for cost savings: LIMOS, a program which controls the optimal dosage of milk of lime within juice purification. Variants of this system are available for both factories operating with filter stations as well as those operating with clarifiers.

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Color Reduction in Sugar House Operation

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Introduction

The production of white sugar with a maximum color of 45 IU is a uniform standard that is aimed for worldwide. The main reasons for this are the increasing share of so-called industrial sugar – sugar that is industrially processed further – as well as the growing quality demands for private consumption that are made above all by large-quantity buyers like supermarket chains.

Meanwhile, it is possible in the beet sugar industry – thanks to considerable improvements achieved in the beet quality – to produce white sugar nearly without problems directly from the thick juice obtained. All process optimizations therefore aim at a crystallization scheme that saves as much energy as possible, and which uses B-sugar – partly or completely – as crystal seed for A-sugar without melting. C-sugar affination has been given up in many cases in order to avoid an increased non-sugar recirculation. It must still turn out under the changed economic marginal conditions whether the resulting, slightly higher sugar content in the molasses will also be accepted in the future.

In sugar cane processing, the developments also yield increasingly better raw sugar qualities (1, 2), but a direct production from concentrated syrup has not been achieved yet. In view of the changes in the world sugar market as a result of the production decrease within the European Union, however, the interface between the cane sugar factory as raw sugar supplier and refinery as white sugar producer and distributor is increasingly gaining importance. The decisive challenge in the further development of cane sugar processing will certainly be the direct production of white sugar without complete refining in the cane sugar factory, in connection with the production of ethanol.

The main emphasis in the development of processes for direct white-sugar production from sugar cane is on the improvement of the juice quality. A further possibility and also necessity to reduce the sugar color consists in the optimization of the crystallization conditions and the connected sugar house operation.

Color in Sugar Crystals

Color Identification

A current detailed survey of the subject "Color formation and removal" was prepared by Bourzutschky (3). With regard to crystallization, especially those examinations are interesting which classify the colorants according to their molecular mass and analyze their behavior in the crystallization process. About 20 to 30 years ago, a series of examinations was carried out on this subject in the beet sugar industry. The paper of Shore, *et al.*, (4) supplemented by Broughton, *et al.*, (5) shall be stated here as the most comprehensive one. From the extensive results of a research program of British Sugar, some essential aspects will be interesting for the following considerations:

When examining the colorants that determine the sugar color, it was distinguished between "external" and "internal" colorants. The external colorants were obtained by washing the sugar in alcohol. The colorants and their molecular mass distribution were analyzed by means of gel chromatography. The internal colorants mainly consisted of high molecular mass colorants, similar to molasses, whereas the external colorants mainly consisted of components with a low molecular mass similar to standard liquor. From this fact it was concluded that colorants with a high molecular mass are preferably inserted in the crystal lattice. There were also other authors who drew similar conclusions for beet sugar production (6) as well as for cane processing (7, 8, 9, 10).

In the examinations of British Sugar, various sugar types were partially dissolved step by step. It was established that A-sugar (white sugar) showed an ICUMSA color value improved by 20% with slight partial dissolution. In the case of B-sugar and C-sugar, 25-30%- dissolution could yield improvements of 80 – 85%. Van der Poel, *et al.*, (8) also described similar results.

Godshall, *et al.*, (7, 8) examined the influence of colorants with different molecular mass and drew the following conclusions:

- 40% to 80% of the colorant remain as in-crystal color with >20,000 molecular mass;
- Higher molecular mass colorants increase during affination, smaller molecular mass colorants are removed;
- High molecular mass colorants increase throughout all refining steps.

For a further discussion of these results it is necessary to consider also the types of adherence / inclusion of colorants. For this purpose it must be distinguished between:

- Inclusions of molecules, preferably with high molecular mass;
- Inclusions of syrup drops in the crystal lattice;
- Adsorption of colorants at the crystal surface;
- Syrup remaining on the crystal surface.

The first two types of insertion of colorants can be influenced only via the crystallization conditions. During the separation of crystal and syrup in the centrifugal, the colorants on the

crystal surface are removed first. By applying a correspondingly high partial dissolution, also incorporated colorants can be removed. However, an improved sugar quality is only achieved if the dissolved portion contains an amount of color that is superproportional compared to the total crystal.

Aspects of a Quantitative Examination

If it is generally spoken of color in the sugar, usually the measuring result for the value according to ICUMSA is understood which means the so-called "color in solution". For determination of this value, rules have been laid down so that there are rarely misunderstandings. If not the measuring results "color in solution" but the process of formation shall be studied, things become considerably more complex. If statements shall be made indicating under which crystallization conditions which crystal quality can be achieved, the effects of various influencing parameters must be known. In addition to the composition of the solution from which crystallization starts, also those parameters are important that influence the kinetics of crystal growth, which also includes the solution components themselves.

For a quantitative examination of the properties of the sugar produced regarding the inclusion of color-determining substances, it is necessary to mentally extend the sugar-water-non-sugar system that is usually examined: A component describing a group of substances is additionally required which is responsible for the color (Schliephake, *et al.*, (12). The designation "colorants" describes all non-sugar substances influencing the determination of the color in solution. Given the relation to the measuring process, it is useful to define the colorants content as follows (12):

$$q_{C/DS} = \frac{Quantity\ of\ colorants\ \ C}{Mass\ \ DS} = \frac{n_C}{m_{DS}} \tag{1}$$

At any point in time, the sugar crystals with the mass m_C contain the quantity of colorants $n_{C,C}$. The colorants content then is:

$$q_{C,C} = \frac{n_{C,C}}{m_C} \tag{2}$$

In each mass element dm of a sugar crystal that is just being crystallized, a quantity of colorants dn_C is enclosed so that this local colorants content is defined as follows:

$$q_{C,C,d} = \frac{dn_{C,C}}{dm_C} \tag{3}$$

This means that the colorants content $q_{C,C}$ can also be determined by integration via the mass. Starting from the seeding point ($m_{C,0}$; $n_{C,C,0}$) the following results:

$$q_{C,C} = \frac{1}{m_C}\left[\int_{m_{C,0}}^{m_C} q_{C,C,d} \cdot dm + n_{C,C,0} \right] \tag{4}$$

This integral describes the composition of a sugar crystal according to the course of crystallization. Since the colorants behave like non-sugar substances, the quantity of enclosed colorants per mass unit will increase in the course of the practical crystallization process. This correlation was examined by Schliephake, *et al.*, (12). The results were described by determining a value C_d from the measured color in solution that can be considered proportional to the local colorants content $q_{C,C,d}$ defined above.

These values are shown in Figure 1 vs. the color in solution, which was converted to water as reference quantity.
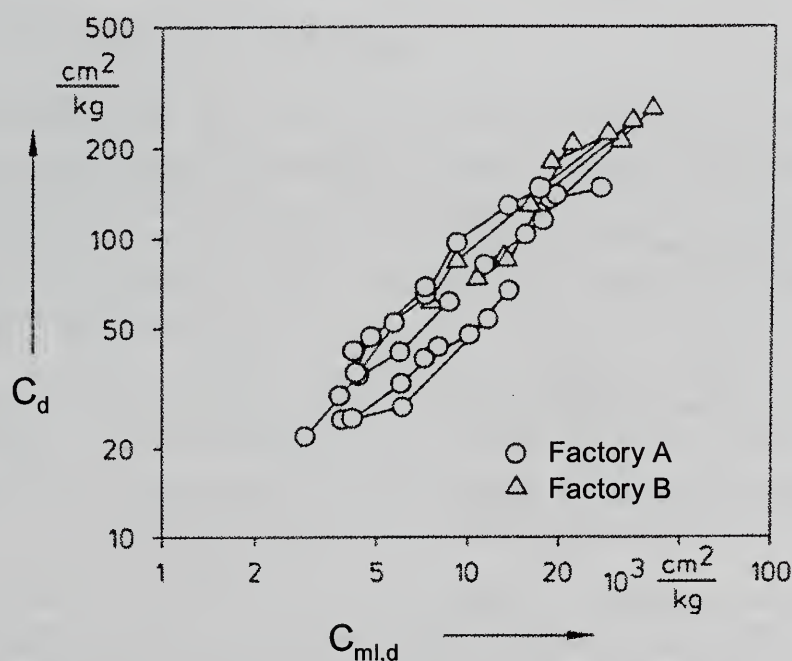
Figure 1. Color in solution (local) vs. color in mother liquor during evaporation crystallization (12).

Color Elimination

The inclusion of colorants and the colorants remaining on the crystal surface are repeatedly referred to the color in the feed liquor or massecuite. From this, a factor for the removal of color during crystallization can be determined. In this connection, various relations are stated. Moodley, *et al.*, (13) reports on results obtained in a pilot plant with different feed solutions. The crystallized sugar was affined so that a possible influence by centrifuging could be excluded. The results can be described within the range 200 – 850 IU with the following equation:

Crystal color = 3.66 + 0.0168 · feed syrup color.

Thompson, *et al.*, (14) describes data from 4 refineries with 4 refined-sugar steps each that can be described by the equation

Color elimination factor = 15.5 + 0.007 · feed syrup color

ranging between 300 – 3000 IU. Figure 2 shows these two relationships.

In addition, the values of Rein (15) were considered reported in connection with examinations of agitators in crystallizers. Here, the sugar color was determined after affination on the one hand and after centrifugal separation on the other hand.
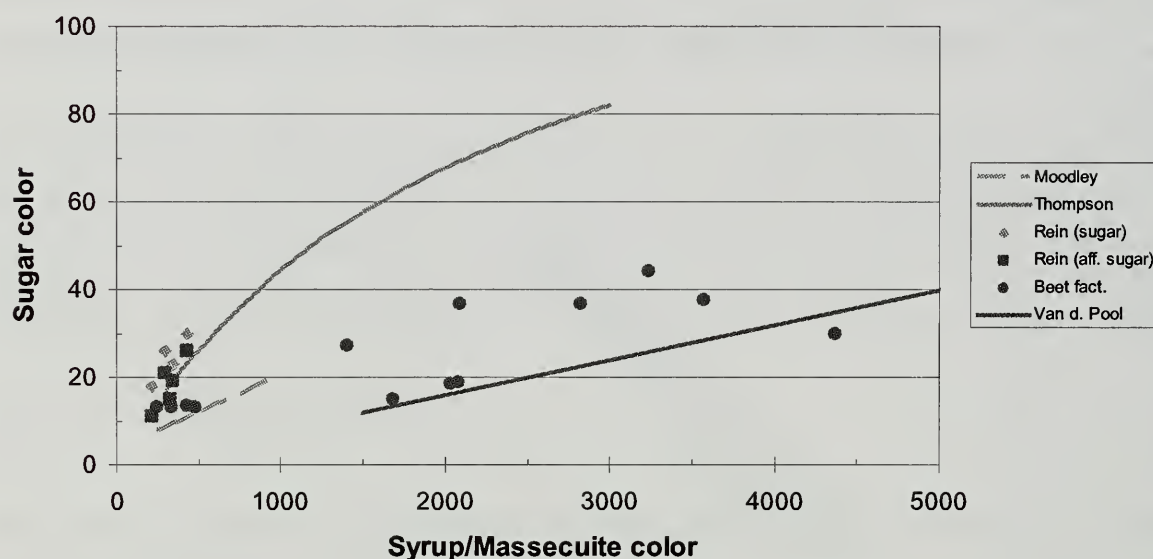
Figure 2.   Sugar color vs. syrup or massecuite color.

For a comparison to the beet sugar industry, existing data from miscellaneous examinations were supplemented. It can be seen that in the range of very low color values, there is an approximation of the color values. This makes sense since the color values from beet sugar factories stand for EG 1 standard quality, where also melted B and C sugars are used as feed solution. The range between 1500 and 4500 IU (EG 2 standard quality) shows a clear spread of the values. For comparison, Van der Poel, *et al.*, (11) states a ratio (Figure 2) of 0.8% (sugar color vs massecuite color) with good crystallization work. A good correspondence with the low color values of the beet sugar factories can be seen.

There are also values for the ranges of B-sugar (batch centrifugals) and C-sugar (color after affination) that can be allocated to the data of Figure 2 by an extended representation on the logarithmic scale (Figure 3). In this case, too, a good correspondence with Van der Poel's ratio can be seen. However, there are only very few data, so that the representation of Figure 3 can only be taken as a tendency.
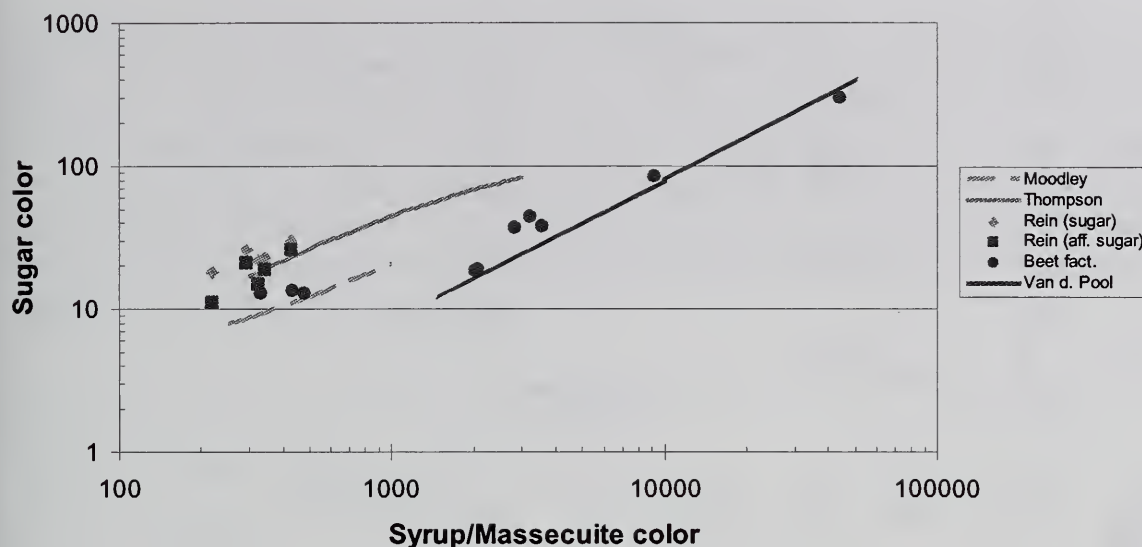
Figure 3. Sugar color vs. syrup or massecuite color – logarithmic representation.

Influences of Crystallization Conditions

The correlations described so far refer to the influence of syrup composition. The inclusion of colorants, however, is also influenced by the crystallization conditions. Basically, it must be distinguished between the inclusion of syrup drops as a result of irregularities such as aggregate formation or insertion into the crystal by a too high supersaturation, and the insertion of singular molecules into the crystal lattice. For the latter one, there is apparently a selection in favor or high molecular mass components.

Rein (15) describes examinations with different agitators and a comparison of manual and automatic boiling. The examinations with agitators as well as those with automatic process control showed a better percentage of color removal and, basically, a smaller increase of color in the massecuite. The increase of color in the massecuite, however, took place particularly at the end of the process during tightening.

Mantovani, et al., (16) presents similar results. He still assumes that single crystal surfaces – those which grow particularly fast – preferably enclose colorants. This, however, is in slight contradiction to the observation indicating that a longer batch crystallization time leads to more color in the crystals. Here, also the simultaneous increase of color in the mother liquor must be considered.

Vaccari, et al., (17) and Mantovani, et al., (18) report on a better sugar quality that can be achieved by complete cooling crystallization in refinery operations as well as in beet sugar factories. The results of Schliephake, et al., (12) from comparison examinations between evaporation crystallization and cooling crystallization confirm this trend (Figure 4).
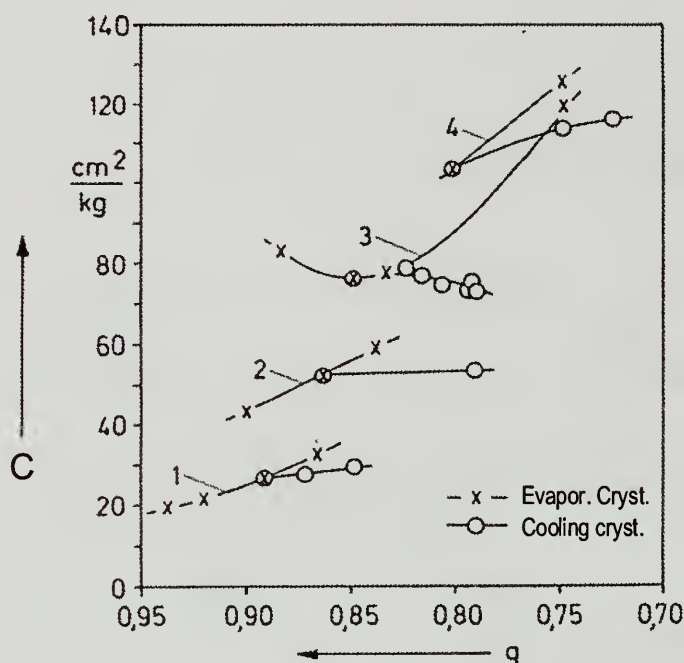
Figure 4. Color in solution vs. purity for evaporation and cooling crystallization (12).

However, a comparison between the results obtained by Vaccari, *et al.*, (17) with the data from Figure 2 shows no clear advantage of cooling crystallization (Figure 5). It must be considered as well that a pure cooling crystallization has a lower yield and, thus, the integration described above will be smaller compared to evaporation crystallization. Particularly the last section – in the range of low purity corresponding to high color in the mother liquor – is missing in the cooling crystallization case.
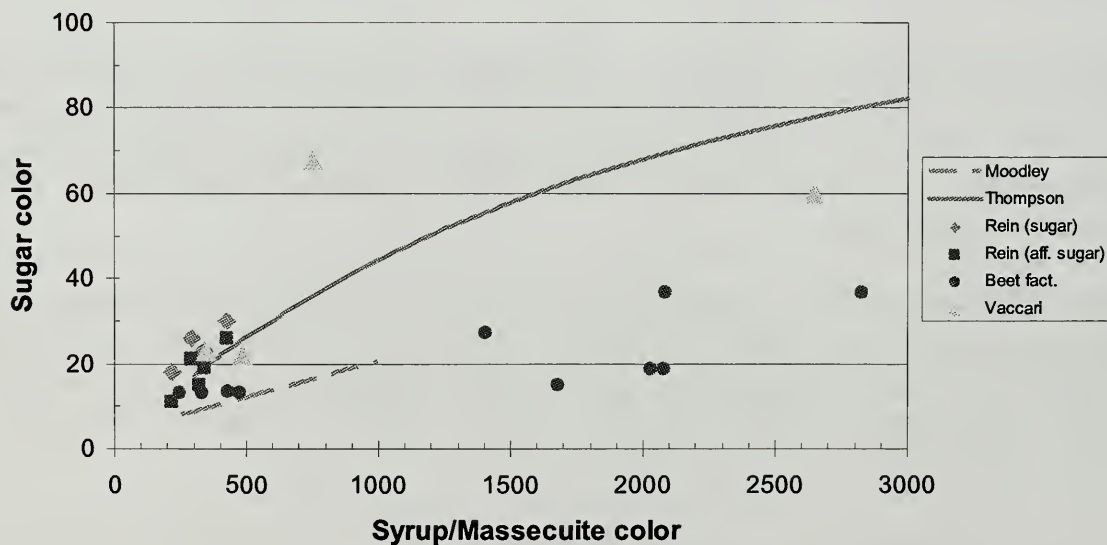
Figure 5. Sugar color vs. syrup or massecuite color including data for cooling crystallization of cane raw sugar.

Therefore, cooling crystallization can primarily serve as a tightening substitute in batch pans. In addition, an increased yield can be obtained by recycling of the syrup in the range of lower temperatures, since in this range the reformation of colorants can be avoided.

Influence of Non-Sugar Recirculation and Seeding Procedures

As described, the concentration of color in the feed solution exerts an essential influence on the sugar color, and non-sugar recirculation is a decisive influencing variable for the color of the final product. Kwok (19) stated data for color and purity of the main products of the sugar house of a cane sugar factory for the production of SVLC (super very low color) sugar. These data were retraced in a mass balance including color balance. This showed a very good correspondence of the color values stated for the sugars (A, B and C) with those calculated, if starting from the non-sugar-proportional color values. Figure 6 represents the calculated color values as well as the color quantities.
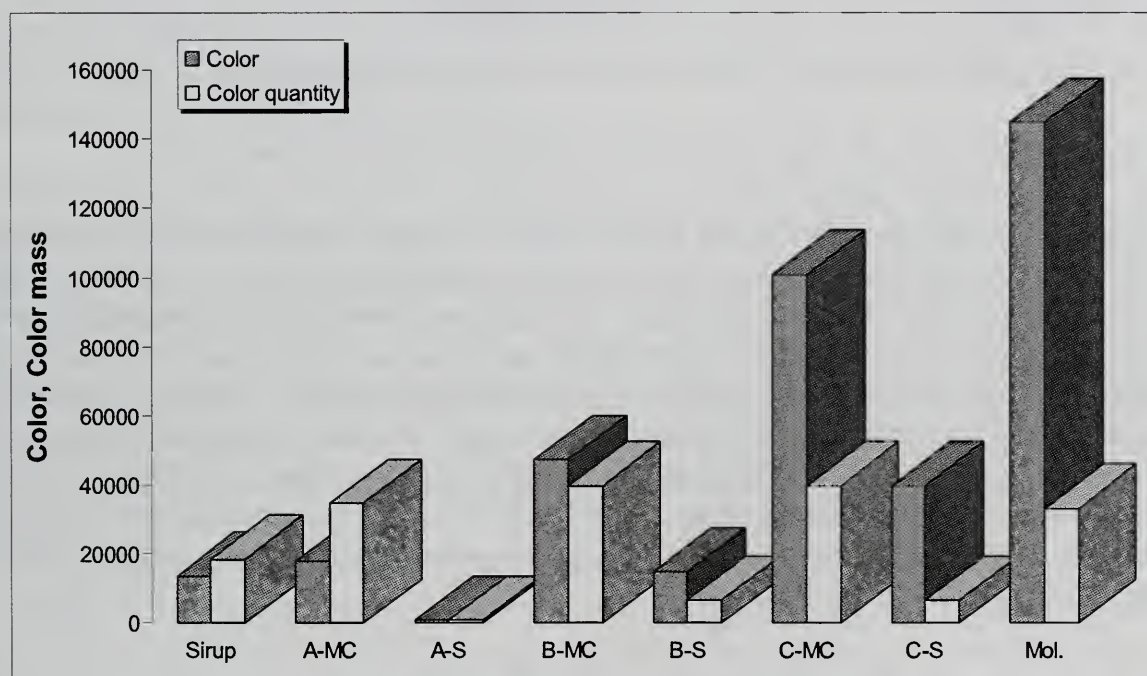
Figure 6.   Calculated color and color quantity according to data from Kwok (19).

Although the second case examined by Kwok of syrup treated by ultrafiltration shows a considerably lower color level, the process only changed by an increase of the raw sugar purity. Therefore, the high non-sugar recirculation remains nearly unchanged.

The color balance also reveals the individual contributors to the sugar color. The percentages of color for the A-massecuite are shown in Figure 7 for both of the cases described above. The values for color increase were determined with the aid of the color balance. The high non-sugar recirculation mainly results from the inefficient separation of sugar and mother liquor in the centrifugals and only to an insignificant degree from the colorants enclosed in the crystal lattice.
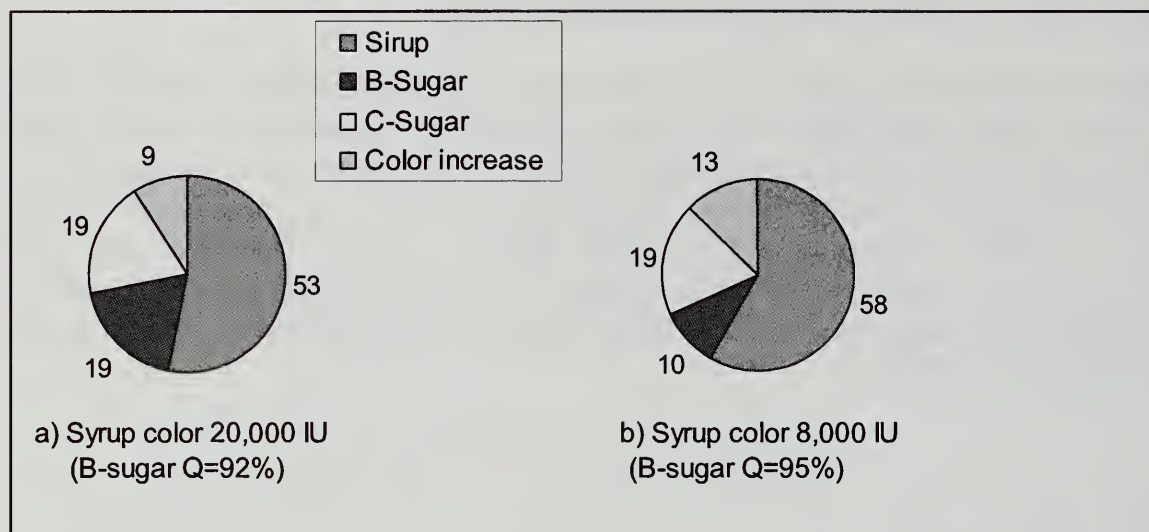
Figure 7.   Contribution to A-massecuite color according to data from Kwok (19).

For both of the cases described it is stated that B-sugar is used as crystal seed for A-massecuite. However, the B-sugar still contains considerable syrup remainders with the purities stated. With a color elimination of 2.5% - 3% exclusively in the A-massecuite-crystallized sugar and in the B-sugar, the resulting color values in the A-sugar can be balanced in both cases. This means that B-sugar contributes with approximately 55% - 65% to the color of the A-sugar. A more exact calculation would require data about the remaining syrup film on the crystal surface and the color elimination by partial dissolution during mingling of the B-sugar. Figure 8 gives an example to illustrate this effect. Partial dissolution leads to a superproportional color decrease, as described above, which reinforces the influence of the percentage decrease of the crystal seed share on the resulting color in the A-sugar.
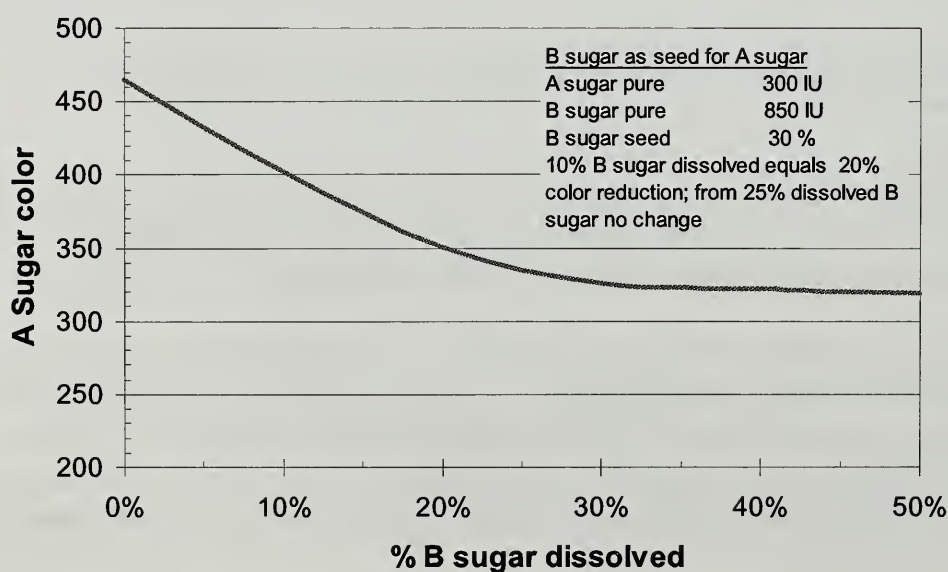
Figure 8.   B-sugar as seed for A-sugar.

This example shows that especially the mingling of sugar as crystal seed must be carried out under strong control in order to secure a constant quality.

A further improvement of the color in the A-sugar on the basis of the examples shown can only be achieved if the following measures are carried out:

- Melting of the B-sugar
- Production of a separate A-seed magma
- Optimization of centrifugal operation for a better product quality

For optimization of the operation of continuous centrifugals, an example is given in Figure 9, which illustrates the influence of throughput and wash water quantity on the quality of the C-sugar produced (beet sugar factory). A disadvantage of a higher wash water quantity and a lower throughput might be a higher purity of the molasses. However, this effect can be reduced by good crystallization and optimized screens.

The measures stated above result in a recirculation of products with a higher purity. This entails the disadvantage that a higher decrease of the purity in the individual crystallization steps is required to reach the same molasses purity again. A possibility for equalization is the efficient separation of green syrup and wash syrup in the centrifugals in order to reach the highest purity possible in the wash syrup. Another possibility for a yield increase without an additional crystallization step is the cooling crystallization as stated in chapter 3. However, the possibilities can only be analyzed more exactly, if there are more data on the color in all crystallized sugars (A, B and C) without the influence of centrifugal separation.
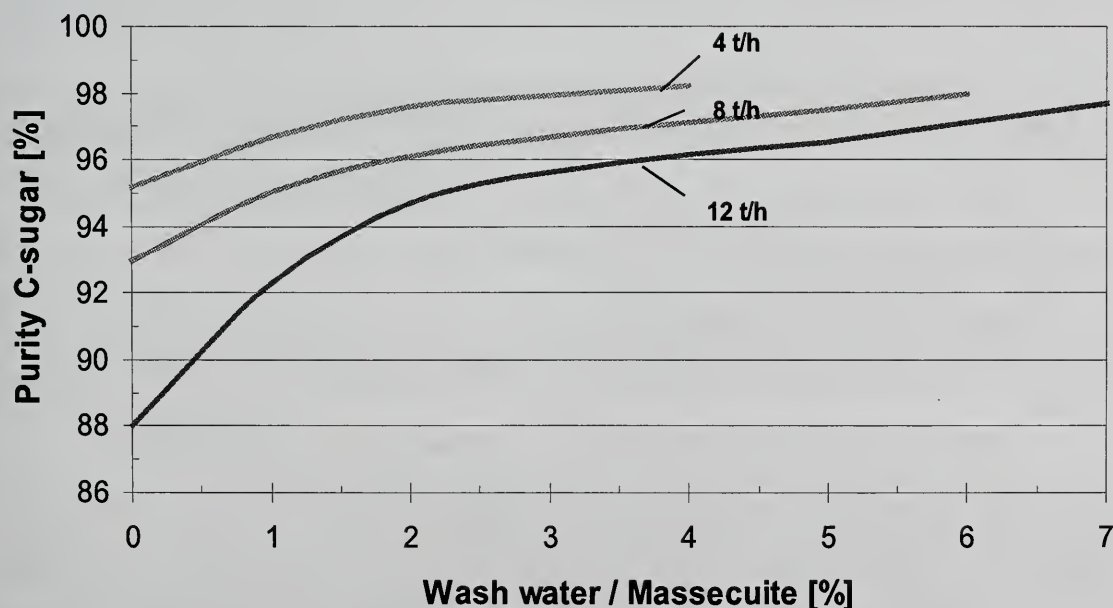
Figure 9. Purity of C-sugar vs. wash water and massecuite quantities (beet sugar factory).

Conclusions

The production of high-quality sugar in a cane sugar factory depends on the availability of syrup with the lowest-possible color as well as, to a decisive degree, on the process in the sugar house. To come to this statement, numerous findings of miscellaneous authors from the beet and cane sugar industry, as well as the sugar refinery industry, were collected and compared to each other.

The concentration of colorants in the mother liquor plays a decisive role for crystallization. For the inclusion of colorants, apparently molecules with a higher molecular mass are preferred. The concentration of colorants in the sugar is a result of an integral process enclosing the colorants according to the respective concentration in the mother liquor. Since also syrup drops can be enclosed and simultaneously the conditions of crystallization have an influence, the overall process is a very complex one. It is important to distinguish between crystallization and the downstream separation in the centrifugal which always leaves a more or less distinct syrup film on the crystal surface.

Color balances allow to illustrate the process well and to determine and evaluate possible potentials for improvement. To this end, however, additional detailed data are required in practical operation.

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Simulated Multiple Effect Evaporation of Clarified and Decolorized Sugarcane Juices and Accelerated Storage Tests of the Syrups: Evolution of Color and the Behavior of Carbohydrates and Related Compounds

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Abstract

Adjuvants were evaluated for potential to either promote or inhibit the formation of color. It was noted during preliminary tests that mixed dithiocarbamate (mDTC) mill biocide can promote non-enzymic color formation. It is known that sodium bisulfite (NaBS) will inhibit browning, but residual sulfite in sugar is undesirable. N-acetyl cysteine (NAC), a modified amino acid containing a sulfhydryl moiety was tested as an FDA approved alternative.

Cleaned, whole stalk sugarcane juice was clarified by hot-liming, and the mud was coagulated via application of conventional flocculant. Once filtered, the sparkling light yellow clarified juice was split into two portions.

Samples of the first portion were either left as-is (control) or treated with mDTC at 20, 50 and 100 mg/kg, respectively, on dry solids (DS) and NAC at 94 mg/kg DS. The samples were subjected to a multiple-effect evaporator simulation (MEES) whereby the juice was brought to 24% DS at ambient pressure, then under vacuum to 50 and 67% DS at 85 and 65 °C, respectively.

The second portion was decolorized via passage through carbon and a mixed bed ion exchanger. Samples of the resulting clear (white) liquid were either left as-is (control) or treated with mDTC at 20 and 100 mg/kg DS, respectively, NAC at 94 mg/kg DS or 60 mg/kg DS of NaBS. These samples were subject to an identical MEES.

Syrups from both clarified and decolorized juices were subject to an accelerated storage test (AST) whereby they were incubated at 95° C for 8 hr with periodic sampling.

For clarified juice, there was little change in color across the MEES. In several of the cases, the color increased slightly across the 1st and 2nd "effects" and decreased across the 3rd. Decolorized juices were less stable, mDTC applied as cited increased the color formed relative to the control; this effect was proportional to the dose. Samples made to contain NAC or NaBS demonstrated inhibited color formation amounting to -24 and -56%, respectively, of the color formed in the control. The most significant increase in color in all cases occurred across the first "effect".

AST results of the different MEES syrups were interesting. The decolorized juices all increased in color, but the effects were different from those in the MEES, with mDTC levels 1 and 2 registering color increases of only 86.2 and 75.1%, NaC 73.2%, and NaBS 72.34% of the control.

Introduction

There are two major barriers between sugarcane and a profit made on sale of product sugar. The first, of course, is getting the sugar out of the cane with minimal loss. Though small when compared to sucrose losses to filtrate and molasses, losses of microbial[1,2,3,4] and chemical origin are well documented[5,6,7,8] and a review of sucrose inversion is given by Madsen.[9] The second, intimate with the first, is the colored material that either enters a mill with the cane, or that which forms as a consequence of the production of raw and refined sugar.

There are two schools of thinking with regards to color (1) removal and (2) inhibition. Removal of color has certainly enjoyed the lion's share of attention through recent history; it generally involves the use of membrane filtration and ion exchangers, viz. White Sugar Mill (WSM)[10] process. These methods are effective, but have some problems when implemented at industrial scale with continuous operation. Problems include clogging or fouling of membranes,[11] leading to frequent cleaning and regeneration of adsorbent materials, viz. regeneration of ion exchangers with acid, caustic, and brine. The latter requires refrigeration (to avoid inversion over cationic resin) which can be expensive, and the large amounts of water used to sweeten-off the resin. This sugar-laden water can create issues with sucrose loss and, if recycled, microbial depredation and the use of steam for re-evaporation.[12] Further, regeneration of ion exchangers, by necessity, produces a large amount of effluent containing salts, sugar, and colored materials which can cause environmental damage.

Inhibition, on the other hand, could lead to a more efficient means of color reduction in product sugars. In principle, the idea is to disrupt, out-compete, or otherwise nullify the path of the color forming reactions that can take place during processing. Unfortunately, in order to undermine color formation, it is necessary first to know the limiting steps in the chemistry involved.

Generally, non-enzymic browning processes are grouped as follows, (1) caramelization, (2) Maillard reaction, and (3) hexose alkaline "degradation" (HAD). Since cane processing does not dwell in the alkaline region, HAD will not be discussed in detail.

Caramel, the "simplest" case, is best described as a series of events beginning with the establishment, at pH>7.0,[13] of an equilibrium,[7] between glucose, fructose, and mannose. From here, fructose is dehydrated to yield 5-hydroxymethyl-2-furfural (HMF).[13,14,15] The HMF is cleaved to yield one molecule each of levulinic and formic acid.[16] The accumulation of the organic acid products lowers the pH. This increases the catalytic activity and, with it, the rate of sucrose inversion and the dehydration/decomposition of the product reducing sugars.[17] At increasing acidity and decreasing water activity, viz. evaporation, a heterogeneous polymer forms which consists of up to 15-28% of difructose dianhydrides.[18]

During this process, the polymerization of some of the HMF also occurs. HMF cannot self-condense,[19] but, it can condense with other carbonyl compounds present in the system.

It has been demonstrated that reducing sugar may experience scission leading to the production of reactive α-dicarbonyl intermediate products including glyoxal, methyl glyoxal, and dihydroxyacetone,[16] which occur via enolization, reverse-aldol scission[7,16] and fragmentation facilited by free radical intermediates.[20] Although not documented as such, the chemistry indicates that HMF will likely oxidize when heated in the presence of air to yield 5-hydroxymethyl-2-furoic acid, which can self-condense to yield the corresponding polyester.[21]

These compounds can condense with HMF, yielding first fluorescent precursors, then a yellow/brown material (depending on extent of reaction) of relatively high molecular weight known as "caramel". The scent and flavor profile of the mixture is mainly composed of pyranones or maltol analogues formed via cyclization and dehydration of fructose, which is similar to that for HMF, but begins with C6-OH rather than C5-OH cyclization via the carbonyl; Antal, *et al.*, present an excellent review of the mechanism involving the formation of HMF from fructose.[14]

In addition to caramelization, if amino acids, amines, or NH_3 are present, a cascade of reactions known collectively as the "Maillard reaction" can occur with reducing sugar to yield dark brown insoluble polymer, frequently in excess of 20 kDa.[22, 23] The "reaction" is quite general in that reducing sugars will react with practically any amine to yield a multitude of products.

The amine reacts with the open chain carbohydrate to yield an unstable intermediate which dehydrates to yield the corresponding "Schiff base" or imine.[24] Under acidic conditions, the imine is protonated which then promotes the isomerization of an aldose to a ketose (Amadori rearrangement[25]) or vice versa (Heyns rearrangement), to yield in the Amadori case, an amino-deoxyketose.

From here, deamination can occur via elimination of the protonated amine to yield a "deoxyosone" or dicarbonyl compound. The amine can then go on to further catalyze this series of reactions. Noted by Hodge,[24] the amine is eventually integrated into the colored material, but not until the later stages, and thus is called a "pseudo-catalyst" here.

These compounds are the keystone of color formation via Maillard reaction. They are universally reactive and can lead to the evolution of aldehydes from amino acids via Strecker degradation. These aldehydes can condense with HMF, each other, deoxyosones, proteins, and many others, leading to a wide distribution of products. The Strecker degradation is the mechanism by which the amine is finally sequestered. Here the amino acid decarboxylates and deaminates,[26] yielding the aldehyde described above, either NH_3 or an amino ketone[27] and CO_2. These amino ketones then can condense into pyrazines[28, 29] (and many other heterocyclic ring structures) which are responsible for a large fraction of the flavor and odor profile of cooked foods.[27, 30] The ammonia can react as cited previously, or can be removed by distillation. The reactions leading from the Amadori compound through the Strecker degradation[27] are given in Figure 1.

"amino-ketone"   "Phenylacetaldehyde"

1,1'-(3,6-dimethylpyrazine-2,5-diyl)
dipropane-1,2,3-triol
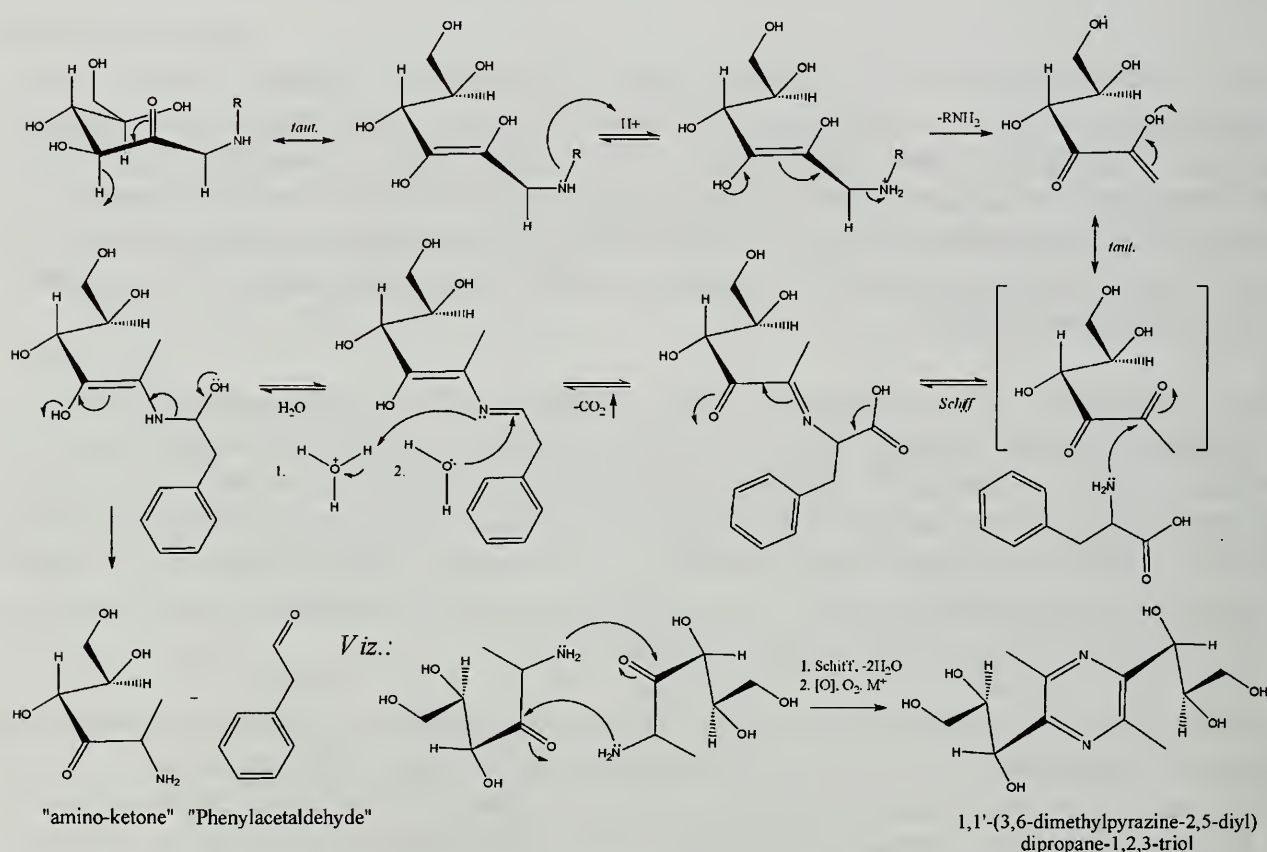
Figure 1. Pathway from the Amadori product though the Strecker degradation with an example of pyrazine synthesis. The key deoxyosone intermediate is bracketed.

In Figure 1 it is seen that steps leading to the dicarbonyl "deoxyosone" intermediate are reversible, and that decarboxylation is permanent. This is key in the formation of the permanent bonds that can lead to the formation of larger, colored structures. If some agent were introduced to interfere with this intermediate, a significant amount of color formation can likely be inhibited. It was first noted by Hodge[31] that browning reactions were retarded in the presence of bisulfite, and that large amounts of bisulfite completely inhibited the reaction.

Bisulfite will add to carbonyl groups to yield water soluble organosulfite salts. A method of purifying aldehydes and methyl ketones,[32] the soluble bisulfite salts can be isolated

from contaminants, and regenerated by treatment with dilute acid or alkali. The scheme for the bisulfite salt addition to a carbonyl is given in Figure 2.
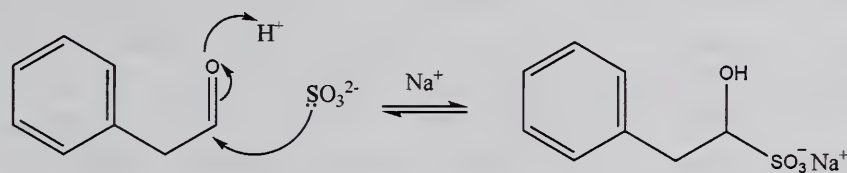
Figure 2. Addition of bisulfite to phenylacetaldehyde.

A portion of sulfite added to wine disappears almost immediately, and must not be used to calculate the active (unbound) sulfite dose lest the wine be insufficiently preserved. It was noted that the bound portion results from reactions yielding carbonyl bisulfite, and further, that this effect has been noted for α-dicarbonyl compounds, including glyoxal and methylglyoxal.[33]

Bisulfite is an effective color inhibitor because it chemically binds the reactive carbonyls, namely the dicarbonyls and Strecker aldehydes. This was observed by Madsen,[34] who noted that the quantity of phenylacetaldehyde volatilized from a mixture of glucose and L-phenylalanine was ~9.5 times lower when inhibited with NaBS; a corresponding reduction in color of ~6.2 times was observed. It was noted that an immediate reduction in color of raw juice was seen on addition of NaBS.

Sulfites are reductive bleaches that are used to great effect through refining processes in countries such as India and Brazil where white sugar is produced directly, viz. the "Dedini Refinado Direto" (DRD)[35] process. Unfortunately, due to an uncommon metabolic disorder[36] known commonly as "sulfite sensitivity", use in the United States of sulfite, a potential systemic toxicant,[37] was regulated in 1986.[38] Although a common standard in many countries, products containing more than 10 mg/kg sulfite must be prominently labeled as such. Because this tends to discourage the consumers, the US manufacturers *do not* use it. However, because it is proven to be highly effective, this study will make use of NaBS as a benchmark.

Following the mechanism of efficacy described for sulfites, it was concluded that other compounds bearing the nucleophilic sulfhydryl moiety might also be effective. L-cysteine (CYS) was tested as an inhibitor in a fructose-L-glutamine (GLN) model.[39] It failed as an inhibitor, leading to greater color and a foul odor. It was concluded that the amino group was participating in the Maillard reaction to a greater extent than the sulfhydryl group was inhibiting it.

N-acetyl cysteine (NAC), a dietary supplement, is used medically as an antioxidant which yields reduced glutathione (GSH) *in-vivo* which aids in detoxification, particularly in the liver, where it has been demonstrated to mitigate lethal acetaminophen intoxication.[40] An intravenous dosage of up to 100 mg/kg (body mass) for 16 hr was well tolerated. The published LD_{50} for this compound is 5050 mg/kg (oral, rat),[41] This compound is *generally regarded as safe*.

NAC has been demonstrated by the FDA to be a satisfactory substitute for NaBS to prevent browning in fresh cut fruit.[42] It was also noted by Friedman, *et al.*, that NAC and reduced glutathione were nearly as effective as NaBS for inhibiting browning of glucose:amino acid mixtures.[43] There was disagreement, however, with Madsen[39] regarding the efficacy of CYS. This might be a difference related to the relatively short time interval tested (120m), or a matrix effect. CYS was found to impart an unsavory aroma at the inhibiting concentration in fruit juices,[44] and thus NAC was found to be superior. In apples and potatoes, CYS was not an effective inhibitor, where again, NAC and reduced glutathione were comparable to NaBS.[45] Following this logic, and the reported efficacy in fruit juices, it was decided to assay NAC for the potential to inhibit browning in sugar cane juice.

Interestingly, there is very little literature describing the use of adjuvants other than sulfite, peroxide[46,47,48] or ozone[49, 50, 51] with relation to color reduction or inhibition during cane processing. One adjuvant, which will be found in process liquor is mDTC biocides, are used in the sugar industry to prevent mill infection and subsequent loss of sucrose. Surprisingly, there is, to the author's knowledge, *no literature* involving the role that mDTC might play in the formation of color during the processing of cane.

It is the object of this work to test the behavior of decolorized process liquor under conditions resembling those witnessed during passage through a "triple" multiple effect evaporator train. In order to further extend the scope of this work, the products of these tests were stored at high temperature such that an assay of kinetic behavior could be made.

Materials and Methods

Calcium hydroxide, 99% (Fisher), phosphoric acid, 85% (Fisher), sodium metabisulfite, 99% (MCB), N-acetylcysteine, 99+% (Sigma-Aldrich), and mDTC was provided as Midland PCS-6001, a 15% w/w mixture of 1:1 methyldithiocarbamic acid, monosodium salt (Vapam[tm]) and ethylenebis [dithiocarbamic acid], disodium salt (Nabam[tm]). In accordance with the sugar industry, these agents were used as-is without further purification.

LCP 85-384 sugarcane was acquired manually (courtesy of Robert Farms); the cut-to-crush time was less than 1 hour. The cane was cut ~10 cm from the ground, topped, and stripped of leaves and sheathing material to yield clean stalks. Clarified juice was prepared as described in the *front-end* of Figure 3. The "effects" in the MEES were carried out using a BUCHI RotaVapor equipped a with digital temperature controller that was verified against a thermometer certified by the National Institute of Standards.

Multiple Effect Evaporator Simulation:

Experimental Design and Conditions
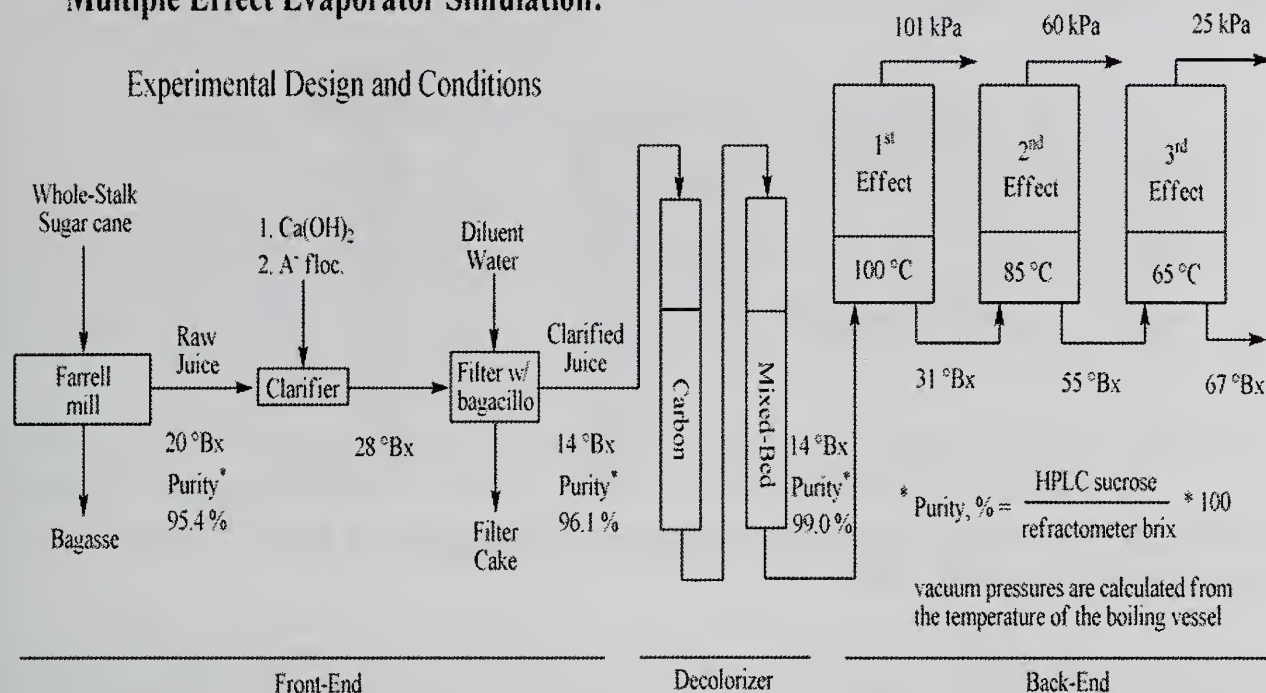
Figure 3. Scheme describing the juice preparation, decolorization, and MEES of clarified juice.

The *decolorizer* section in Figure 1 was comprised of activated carbon and a mixed-bed ion exchanger. The carbon was provided by Calgon. It was washed with hot water to remove the fines and then transferred into a Pyrex[tm] glass column in slurry to provide a 300 mL bed.

The mixed bed was comprised of strong-cation exchange resin in the sodium-form, Purolite C-150 MBNA (brown) and weak-base exchange resin, Purolite A-510S MB (white) in a 1:2.4 ratio. Both resins were regenerated before use with 0.5 M NaOH and brine. It was noted that the anionic resin exhibited a change in color from white to pink when treated with the alkali. Both resins were washed until the rinse was neutral to litmus.

The clarified juice was divided in half; one half was subject to passage through the decolorizer section of the scheme in Figure 1 at a rate of 2.13 BV/hr and 75°C. The decolorized juice was water-white and free of turbidity until the passage of ~16 bed volumes, when color was noticed to increase. Every 2nd L of decolorized juice was transferred to the freezer at -74° C to yield 5 L of ~80 IU composite liquor. These samples were thawed, diluted by mass, and analyzed for carbohydrates, pH, and color.

The clarified juice and decolorized liquors were divided and treated individually as described in Table 1.

Table 1. Sample preparation matrix

Matrix:	Adjuvant:	Adjuvant mg/kg DS:
Clarified Juice	control	0.0
	mDTC	20.0
	mDTC	50.0
	mDTC	100.0
	NAC	94.0
Decolorized Liquor	control	0.0
	mDTC	20.0
	mDTC	100.0
	NAC	94.0
	NaBS	60.0

Each mixture was passed through the *back-end* section described in Figure 1 to yield syrup of approximately 67° Bx.

Each syrup was assayed for sucrose, glucose and fructose via HPLC/DRI using an Aminex HPX-87K column (300 mm X 4.2 mm (ID), 5μm) and 0.01 M K_2PO_4 eluent at 0.6 mL/min. HMF was assayed using reversed phase HPLC and a water:methanol (MeOH) gradient (1:99 to 60:40 methanol:water over 20 min) on a 250 mm X 4 mm (ID), 5μm Burdick and Jackson octadecylstyrene (C_{18}) column with DAD detection (254, 280, and 330 nm). The samples were scanned at pH 7.0 from 200-750 nm, and pH and dry solids (refractometer Brix) were determined.

Each of the syrups was transferred as 10 X 10 mL aliquots into scintillation vials with poly seal caps. The sample sets were incubated at 95° C. Samples were periodically removed and immediately frozen at -74° C. Samples were thawed rapidly, diluted by mass, and assayed for carbohydrates, HMF, pH and color.

Results and Discussion

Multiple Effect Evaporator Simulation (MEES) on Clarified Juice

On clarification, the juice increased in purity (HPLC) by 0.72 % and the color decreased from 9,120 to 7,275 IU, a reduction of 20.2 %. It was interesting that upon addition of the mDTC, there was an immediate increase in ICUMSA color that was not apparent to the eye. This effect was proportional to mDTC dosage.

Table. 2. Initial and final color of syrups prepared from clarified juices on a per-sample basis and against the final control value.

Sample:	Start, IU_s:	Final, IU_f:	Δ IU IU_f - IU_s:	IU_f - Control:
Control	7533	7895	+ 362	0
mDTC 20 mg/kg	8357	8490	+ 133	+595
mDTC 50 mg/kg	8716	8870	+ 154	+975
mDTC 100 mg/kg	10201	8425	-1776	+530
NAC 100 mg/kg	6194	6911	+ 717	-984

From Table 2 it is observed that there appears to be some anomalous effect resulting from excessive dosages of mDTC. Figure 4 displays the color dynamics of this system.

Evaporation of Clarified Juice: Color, IU
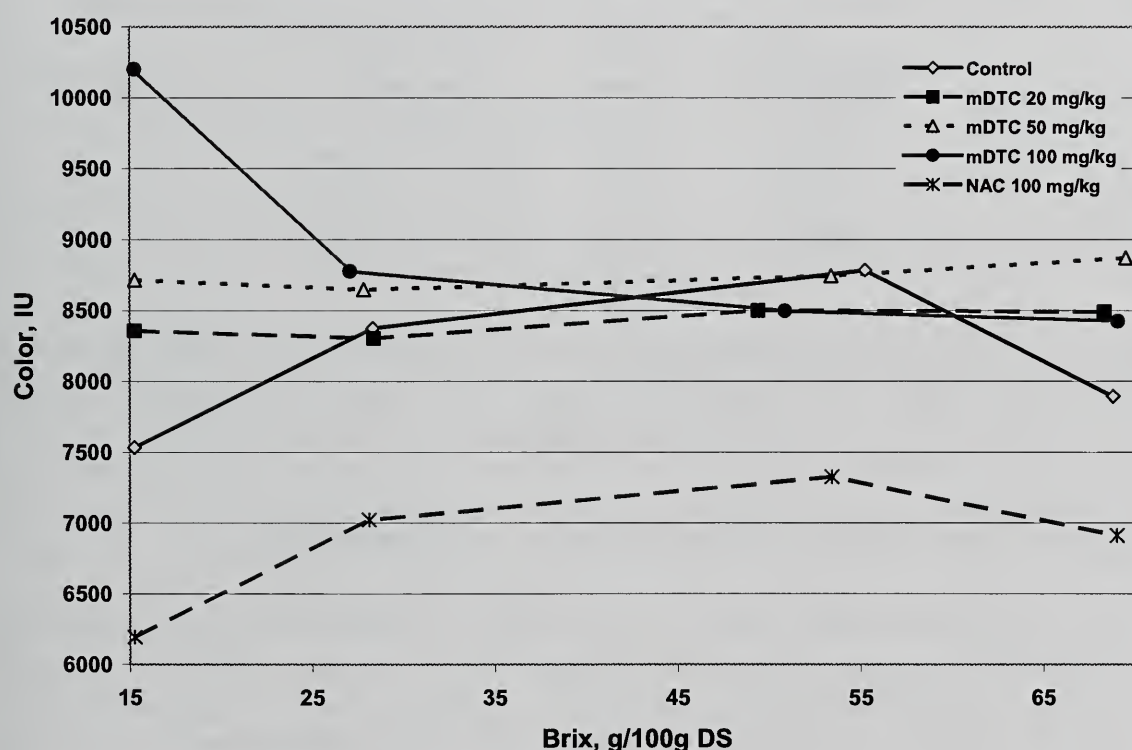
Figure 4. Color at various stages of evaporation.

It can be seen that in Figure 4, there is a net increase across three effects in the control, but, in the sample containing 100 mg/kg mDTC, an anomalous decrease in color was observed. It was noted by Madsen[52] that that 100 μg/g of mDTC could only contribute ~3.0 IU (up-front) to the total; this is negligible, by comparison, to the color increase on dose that is observed.

Unfortunately, the bulk of the literature involving color and evaporation seems dominated by studies conducted in beet mills where operation occurs at pH 9-7. [53] de Bruijn, *et al.*,[54] described another beet evaporator train where the color through the first three effects

appeared similar to the results given in Figure 4. The sample fortified with NAC behaved exactly like the control, save a consistent negative bias of 1384 ± 65 IU. In order to attempt an explanation of the observed color values for the mDTC fortified samples, the pH is given for each effect in Figure 5, below.

Evaporation of Clarified Juice: pH
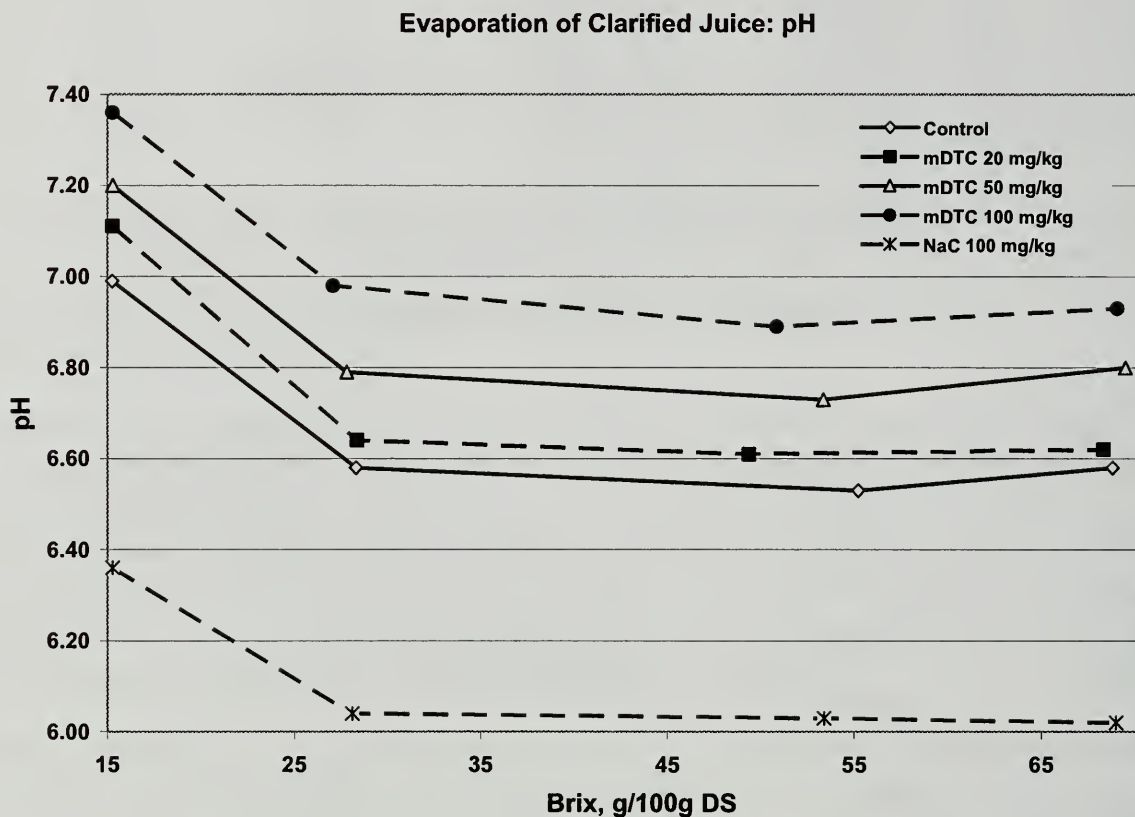
Figure 5. pH, measured at 25° C, of clarified juices under evaporation.

In all cases, the pH drops ~0.40 ± 0.05 from the initial conditions through the first effect. The initial conditions, it appears, are quite different. The data suggests that the rate of pH drop is somehow independent of the initial pH. There appears to be a near linear relation between dosage of mDTC and the starting pH, viz. the mixed dithiocarbamate product is quite alkaline (a 0.26 M solution has a pH measured at 10.93), and when applied at 100 mg/kg puts the starting condition into the bottom of the pH range used for beets.

It does not remain there long. At 20° C, and pH 5, 7, and 9, one of the components of mDTC, Vapam[tm] has half-lives of 23, 180, and 46 hr, [55] respectively. But, it was observed that solutions of surrogate syrup (sucrose, 95, glucose, 2.5, and fructose 2.5 g/100g DS, respectively) fortified with mDTC (500 µg/g DS) demonstrated a half life of only ~9.2 *minutes*[52] (see appendix A) when processed at 100° C. All the while, the pH will be dropping not only as a consequence of organic acid production from reducing sugar, but from hydrolysis of the dithiocarbamate to yield mainly methylamine and CS_2 with traces of elemental sulfur and dimethylthiourea[55] (Nabam[tm], the other component of mDTC, would yield ethylenediamine and CS_2).

There will certainly be some, perhaps large, percentage of the product that has hydrolyzed by photochemical or thermal means. Normally, the free amines would increase the pH, but at pH 7.5-7.0 they are likely to react very quickly (much faster than an amino acid) with phenolic materials at room temperature in the presence of air (especially if Fe^{3+} is present) to cause a large increase in color up front. In the absence of a reducing agent, these complexes will hydrolyze as the pH changes. It is possible that transient "Maillard" chromophores might form via a reaction that is reversible until marked dehydration and/or scission occurs.

The color for the mDTC fortified samples remains relatively static from the first through the third effect to syrup, with a very slight increase in pH seen across the third effect which does not seem to account for the color drop seen for both the control and the sample fortified with NAC.

Table 3. Loss of sucrose on evaporation of clarified juices; dosage is on dry solids.

Clarified Juice Adjuvant:	Syrup pH:	Δ Sucrose g/100g DS
control	6.58	0.572
mDTC 20 mg/kg	6.62	0.000
mDTC 50 mg/kg	6.80	0.000
mDTC 100 mg/kg	6.93	0.000
NAC 100 mg/kg	6.02	1.026

As seen in Table 3, the increased pH resulting from the application of the mDTC prevented a net loss of sucrose across the evaporator train. The control exhibited a 0.572 g/100g loss whilst the sample treated with NAC lost almost twice that amount. It is worth noting that the NAC was able to reduce some colorants up-front resulting in a net reduction of ~1300 IU, against the control and maintained an improvement of ~1000 IU through the evaporator train despite the fact that the quantity of reducing sugar was increasing due to inversion. This suggests that the NAC was operating as a colorant reducer/inhibitor, but that the free carboxylic acid moiety caused too much of a pH drop for its use to be profitable, viz. 1.026% loss amounts to about one-day's value per season or ~$444,709/season · mill (crushing 0.82 million metric tons cane/season at $0.44/kg sugar). Since the rate maxima for the Maillard reaction occurs above pH 7,[56] it appears possible that the lower pH assists the NAC by minimizing the amount of reactive intermediates that are formed from reducing sugar.

MEES on Decolorized Liquor

The bench scale carbon/mixed bed ion-exchange decolorizer was quite efficient, removing on average 97.7 % of the colored materials from the clarified juice of color 7640 IU to provide a liquor with a color of 80 IU. The color began to break through at ~16 bed volumes (BV) which is clearly seen in Figure 6.

Although the washings from the resin during preparation were neutral to litmus, the effluent from the column was initially quite alkaline, viz. pH 10.29. The pH dropped at a

rate corresponding to $9.86 \cdot BV^{-0.105}$ ($R^2=0.9886$). The pH across the decolorizer as a function of BV is provided in Figure 7. From this, it appears that some alkali was likely displaced from the resin (or H^+ removed from the juice) with the adsorption of colored material and/or ash.
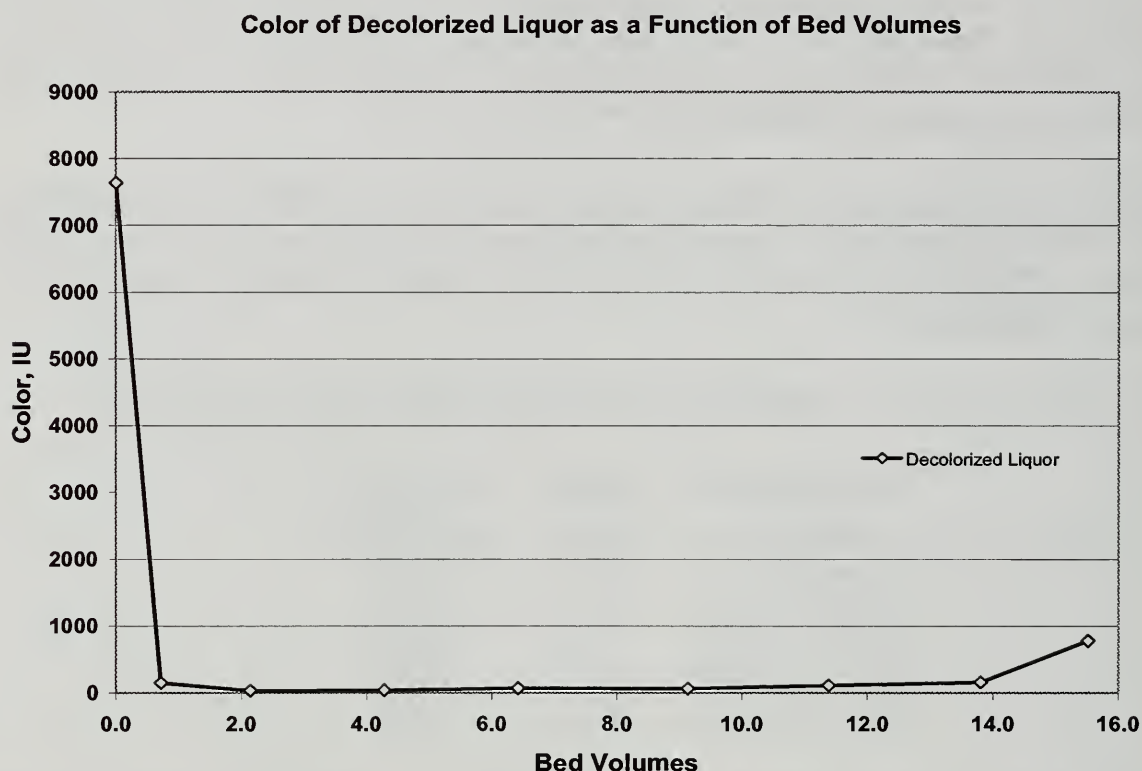
Figure 6. Decolorization as a function of BV.

On evaporation, in Figure 8, decolorized liquors containing mDTC (at the highest and lowest levels tested in the clarified juice) demonstrated a similar elevation in initial color, but to a much greater extent relative to the control, viz. 20 and 100 mg/kg mDTC have initial color of +7.5% and +6.7% in clarified juice where the analogous values for the decolorized liquor were +23.4% and +102.7 %, respectively. The initial decrease in color for clarified juice samples containing NAC was not seen in the decolorized liquor. This supports the earlier conclusion that the NAC is neutralizing some of the colorant material early on; in the decolorized liquor there is little, if any material to interact with, and hence, no observed decrease in color. Relative to the control, NAC and NaBS prevented the formation of 21.0% and 55.8% of color, respectively. The pH values of the liquor fortified with NAC did *not* become sufficiently acidic as to lead to inversion over the control.

Decolorizer: pH vs. Bed Volumes
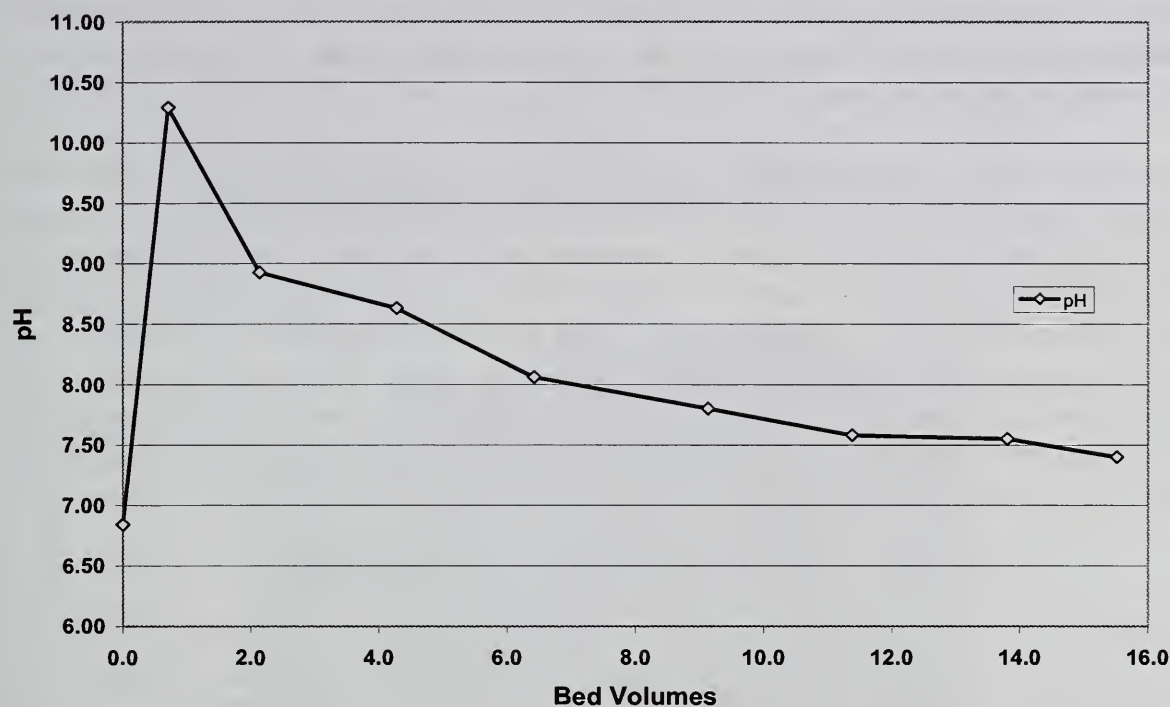
Figure 7. pH across the decolorizer.

Decolorized Liquor: Color vs. Brix, g/100g DS on Evaporation
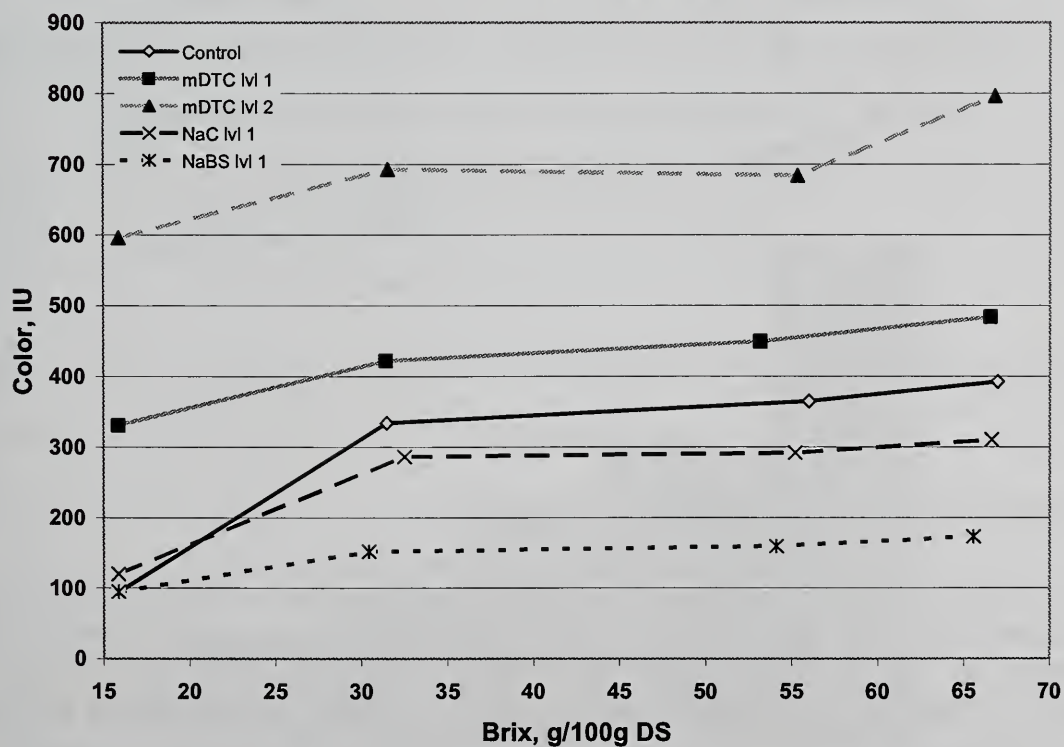
Figure 8. Evaporation of decolorized liquor.

Unlike the clarified juices, the decolorized samples treated with mDTC did not decrease in color from the initial condition even though the pH profiles were similar in shape (Figure 9). This supports the previous conclusion that colored complexes formed early-on were falling apart with the change in pH. But, it also argues against the premise that it was the "complex effect" alone.

The decolorized liquor demonstrated significantly less buffering capacity than the clarified juice, and the effect on pH of the adjuvant reflects this, shifting the initial operating pH regions to 7.5-8.5. This pH is square in the middle of the optimum range where the Maillard reaction to occurs. In light of this, it was concluded that the mDTC contains some initial amount of amine that, even at room temperature, can form both transient and permanent color bodies; the extent of either is favored by pH, viz. "transient" (reversible) at pH 6.6-8.5 and toward "persistent" (irreversible) colorants at lower pH. Higher temperature, solids, and extended time would favor the formation of the "persistent" colorants.

Figure 9. pH of decolorized liquors during evaporation.

All-in-all, if white sugar is the object, then for the mill front-end, it is the final syrup color that matters the most. It is demonstrated in Figure 10 that the overall effect of the tested adjuvants is small in clarified juice, viz. 6911-8870 IU or ±12% of the control value. Figure 11 demonstrates the much more profound overall adjuvant effects that can be seen in decolorized liquor where the color ranges from 176-796 IU or -55.8% to 102.7 % of the control value.

The decolorized liquor fortified with NAC exhibited less inversion than the control. It produced evaporator syrup with a color of 310 IU which is far better than that seen using ultrafiltration and resin, viz. the "New Applexion Process" (NAP) [57] where syrup of 4500 IU was capable of producing 96 IU sugar. In fairness, the NAP was starting with a clarified juice of 17,700 IU, so applying the decolorization percentage achieved in this work with carbon, resin and NAC, we might see a syrup color of ~754 IU.

Evaporation of Clarified Juice: Final IU Color at 66.5 brix, g/100g DS
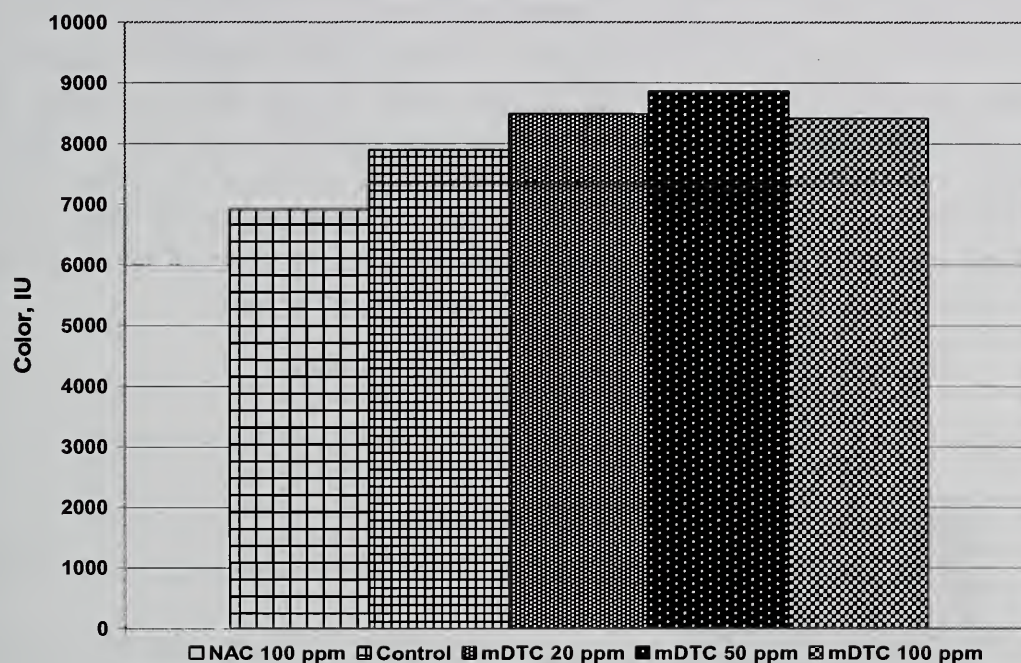
Figure 10. Color of final evaporator syrups made from fortified clarified juice.

Evaporation of Decolorized Juice: Final IU Color at 66.5 brix, g/100g DS
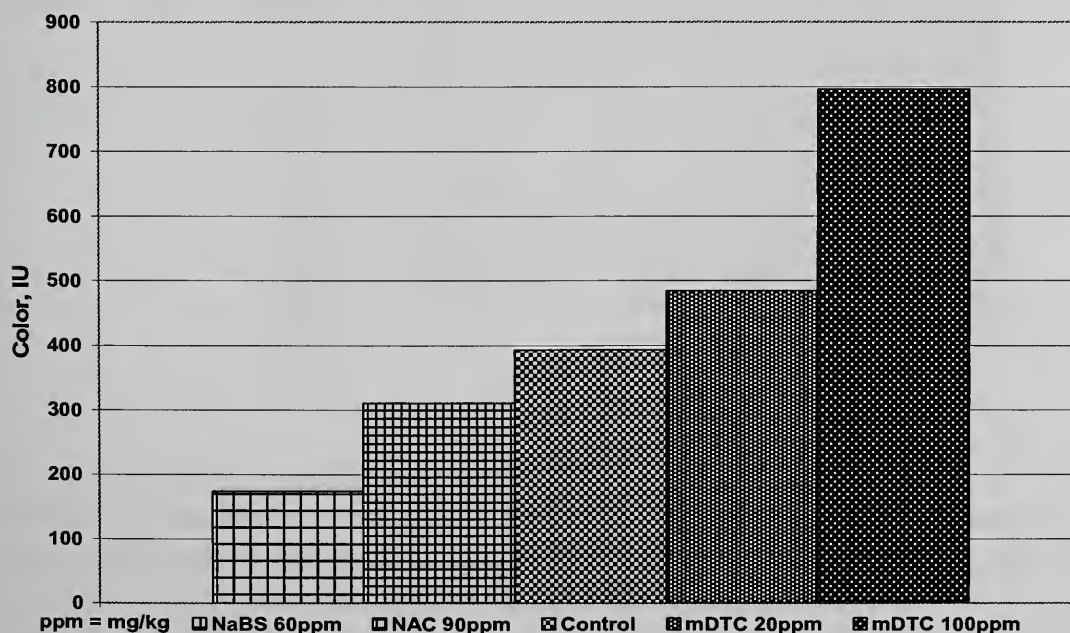
Figure 11. Color of final evaporator syrups made from fortified decolorized liquor.

Accelerated Storage Test (AST) of Decolorized Liquor

In the AST, the clarified juice syrups did little but exhibit a slight increase in color. It is possible that the increases were obscured in the high background color of the samples. The decolorized syrups displayed a curious behavior.

As seen in Figure 12, the color formed on storage appeared to be inhibited by some product resulting from the hydrolysis and subsequent reactions involving the mDTC. During the course of the AST, the control exhibited an increase of color from ~400 to 7213 IU, which was the largest increase and highest ultimate color observed. Relative to the control, NaBS had the smallest increase in color with a final value of 3861 IU (-46.5 %). There is also seen a dose-responsive inhibition of 6221 IU (-13.8 %) and 5417 IU (-24.9 %), respectively for mDTC at 20 and 100 mg/kg DS which is very close to the 5283 IU (-26.8 %) seen for NAC applied at equivalent concentration. It has been noted that some Maillard reaction products can be effective inhibitors themselves,[58] which credits the theory that the mDTC system may be self-inhibiting over time.

Decolorized Syrup AST, Final Color at 7.3Hr, 95-100°C
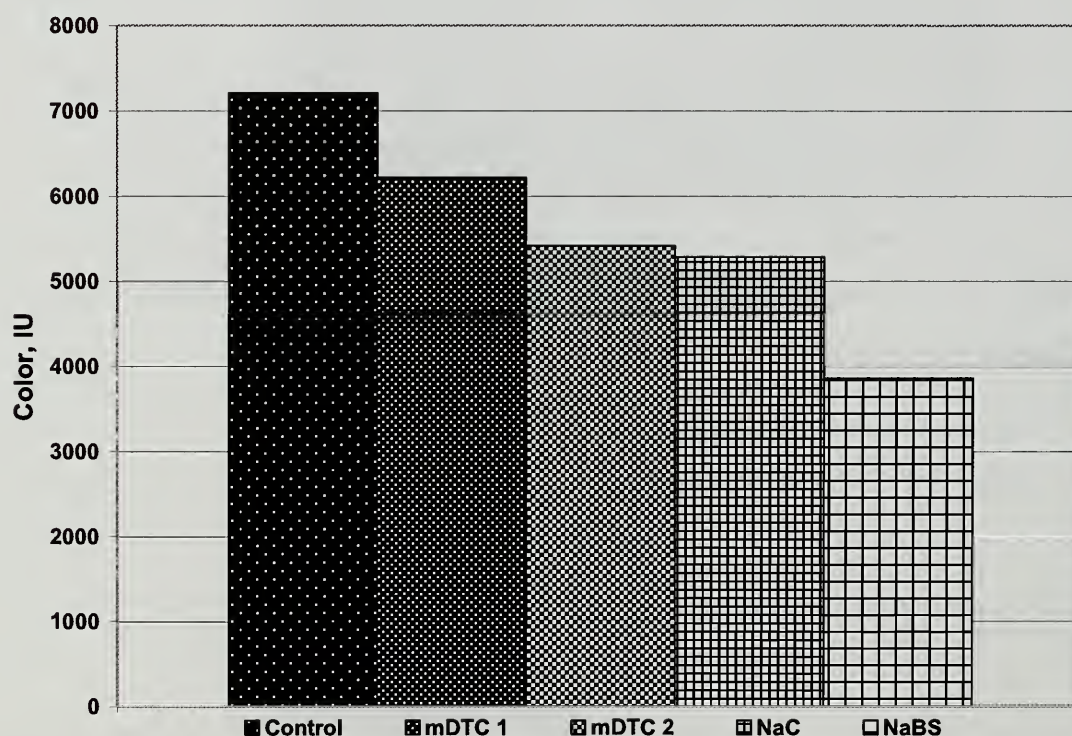
Figure 12. AST of syrups prepared from decolorized juice.

It was also noted, contrary to pure sucrose solutions where the correlation of color to HMF is frequently linear,[59] for all decolorized liquors tested, the relationship was logarithmic, viz. behaving more like a Maillard reaction. The natural logarithm of the color, IU was plotted against the HMF in mg/kg DS on log scale to prepare Figure 13; the worst relation produced $R^2 = 0.9825$.

Decolorized Liquor AST: Relationship of Color, IU and HMF, mg/kg DS

The chart displays the following fitted equations:

$y = 0.4501 Ln(x) + 5.4583$, $R^2 = 0.9975$ — ◆ control

$y = 0.3835 Ln(x) + 5.8699$, $R^2 = 0.9956$ — □ mDTC 20 mg/kg

$y = 0.3705 Ln(x) + 5.9769$, $R^2 = 0.9877$ — ▲ mDTC 100 mg/kg

$y = 0.428 Ln(x) + 5.5686$, $R^2 = 0.9825$ — ○ NAC 94 mg/kg

$y = 0.564 Ln(x) + 4.7665$, $R^2 = 0.993$ — ● NaBS 60 mg/kg

Axes: ln Color, IU (vertical); HMF, mg/kg DS, log scale (horizontal).
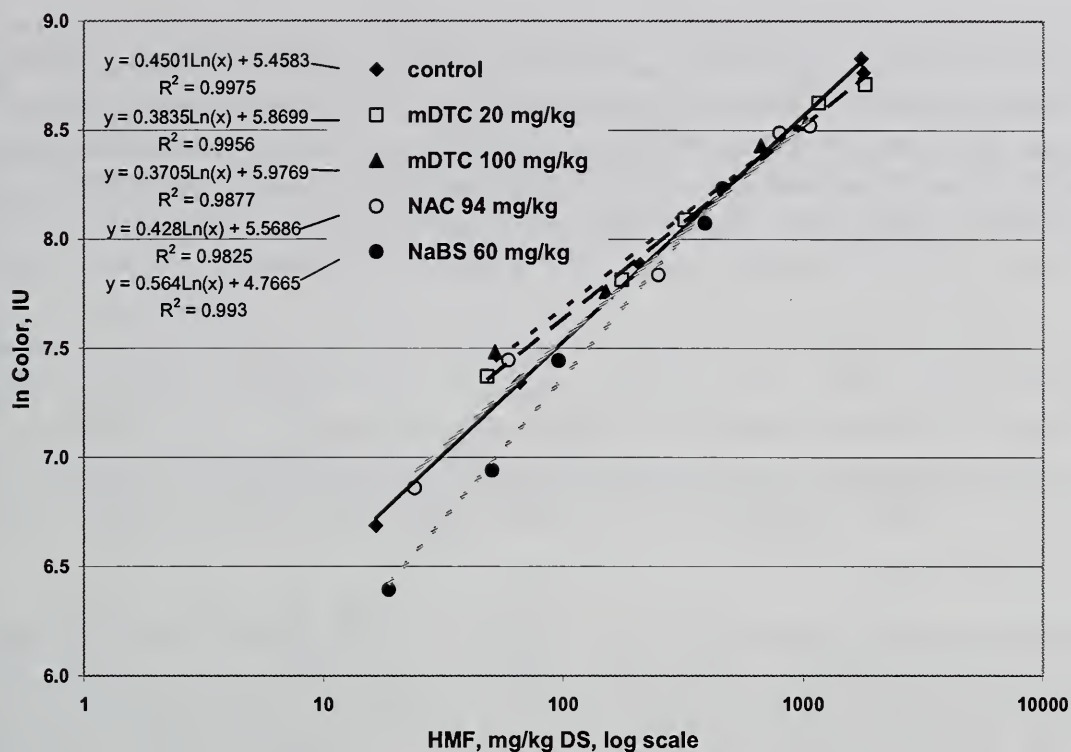
Figure 13. Correlation of ICUMSA color to HMF.

It was noted that the onset of HMF evolution was retarded by addition of mDTC, and that this effect was relative to the dose. The evolution of HMF over time during ASTs of the decolorized liquors is given in Table 5.

Table 5. HMF, mg/kg DS detected in decolorized liquors subjected to an AST.

Elapsed time, hr:	Control:	mDTC 20 mg/kg:	mDTC 100 mg/kg:	NAC 94 mg/kg:	NaBS 60 mg/kg:
0	0.0	0.0	0.0	0.0	0.0
1	0.0	0.0	0.0	0.0	0.0
2	16.5	0.0	0.0	0.0	0.0
3	65.6	48.1	0.0	26.6	18.7
4	208.5	175.1	58.0	66.4	50.4
5	311.5	320.8	176.0	284.0	95.3
6	1774.5	1155.7	882.3	1151.8	388.4
7	1733.8	1804.2	1282.1	1514.9	461.0

It is interesting to note that where mDTC increases the induction time, it does not affect the rate. This leads to a lower final color on AST. The similarity in results for mDTC at 100 mg/kg and NAC at 94 mg/kg are in agreement with the nearly equivalent final color, and it is found, even with NaBS, that the final HMF content and the final color of the mixtures were highly correlated where $R^2 = 0.9066$.

Conclusions

It was concluded that NAC is an inhibitor that is both effective and safe. To avoid the possibility of inversion, care must be taken with its use in terms of dosage and the pH resulting from application. This is particularly true with decolorized liquors where the buffering capacity appears to be nil. While not as effective as NaBS, it does not appear to cause any risk of the illness associated with sulfites and should prove an effective agent, particularly in the stabilization of decolorized liquors toward production of white sugar directly.

It may be possible to modify the molecule in order to nullify the carboxylic acid moiety, thus eliminating the bulk of its acidic capacity, viz. selective reduction to the corresponding N-(1-Hydroxymethyl-2-mercapto-ethyl)-acetamide. The mechanism of operation was not disproved, which suggests that other agents of this kind may prove to be equivalent or better inhibitors of the intermediates involved with non-enzymic browning.

mDTC hydrolyzes quickly ($t_{1/2}$ = 9.2 min. at 100 ° C) to yield free amines which appear to react at room temperature, perhaps with phenolic compounds and reducing sugar, likely both, to yield labile colored complexes. These complexes may be hydrolyzed upon change in pH, and may or may not, depending on the conditions of temperature, solids content, pH, contact time, participate in the evolution of persistent color bodies.

The reactions of mDTC breakdown products, while initially leading to an increase in color over the control, exhibited inhibiting potential on storage at high temperature (95° C) for extended periods of time. Since the agent(s) in question should only be present in amounts significantly smaller than the amount of parent compound applied, the inhibiting compound should be highly active, and could prove ultimately to be a more cost effective alternative to NAC.

Surprisingly, decolorized liquor behaves more like a modeled Maillard reaction where the relationship between HMF and color is logarithmic; this is contrary for caramels where HMF correlation with color is linear.[59]

The breakdown of mDTC increases the period of induction leading to the onset of HMF evolution, but not of color. Color in systems containing mDTC evolves constantly from start to finish indicating that while highly correlated with color, HMF may not, in fact, be an ultimate precursor early-on in these systems. HMF in samples treated with NAC behaved similarly to samples treated with mDTC at equivalent concentration, but the ultimate color was lower. The sample treated with NaBS developed HMF with an induction period similar to NAC or mDTC, but after that, the rate was approximately 1/2 of that observed for the other systems.

Acknowledgements

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Appendix A.

UV-VIS Spectrum of mDTC in Syrup Surrogate at 22.25 °Bx, 100 °C
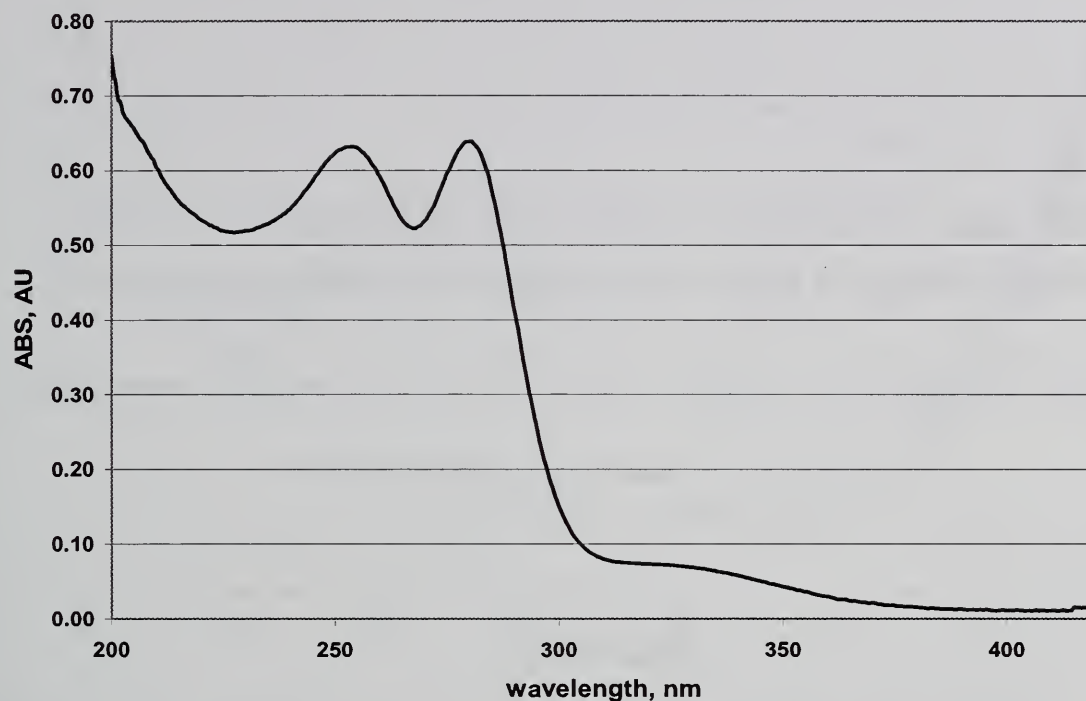
Figure 1A. UV-VIS spectrum of mDTC at 50 mg/kg. Note shoulder at 342 nm.

Effect of mDTC Dosage on Absorbance at 420nm in Aqueous Solution

$y = 0.0299x$

♦ 12 °Bx
——Linear (12 °Bx)
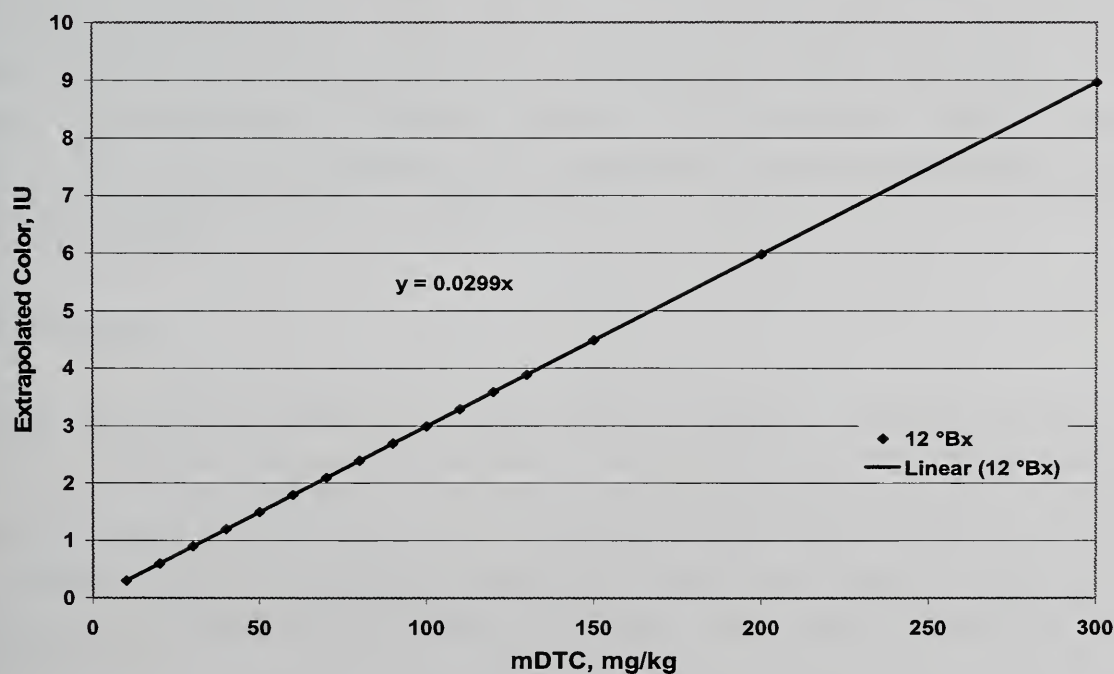
Figure A2. Linearity was established from absorbance at 342 nm; values at 420 are extrapolated from a solution of mDTC at 22 191 ug/g mDTC.

Behavior of mDTC in Syrup Surrogate at 100 °C

$$y = 100e^{-0.1118x}$$
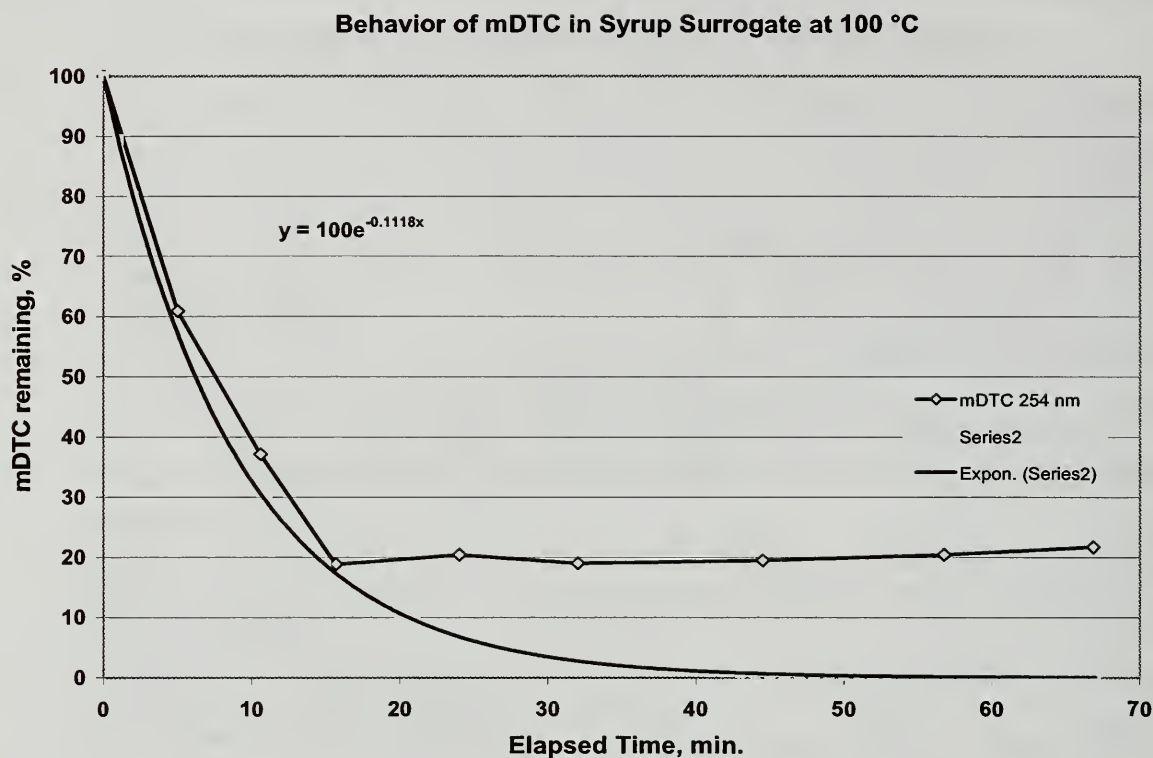
Figure A3. mDTC destroyed over time. The $t_{1/2}$ is ~9.2 minutes and competing materials at 254 nm begin to interfere at ~ 15 min. The kinetic plot is for comparison.

Effect of Enzymes on Color, Turbidity and Total Polysaccharides in Sugarcane and Sugarbeet Juice

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Abstract

Control of color, turbidity and polysaccharides is important in sugarcane and sugarbeet processing. Controlling these parameters as early as possible in the process will provide benefits to the manufacturer in terms of lowered use of processing aids, improved filtration, better sugar recovery, and higher quality products. Twenty-eight commercial enzymes with targeted functionalities were examined for their ability to reduce color, turbidity and/or polysaccharides in raw beet and raw cane juice. Juices were treated with 500 ppm enzyme for 30 min at 50° C and monitored for reduction of color, turbidity and total polysaccharide content. For cane juice, enzymes with hemicellulase, cellulase, xylanase, and glucosidase activity were the most effective. For beet juice, enzymes with hemicellulase, pectinase, xylanase, and glucanase activity removed significant color or polysaccharide. Several enzymes also decreased turbidity in cane and beet juice.

Introduction

Sugarcane and sugarbeet constitute the main sources of sucrose. Sugarcane is grown in warmer tropical and subtropical climates and sugarbeets are grown in colder climates. Although these two sources are vastly different, the crystalline sucrose end product from each is the same. The purification process for each system is also different, but the initial juice from which sucrose is to be extracted in both is turbid and dark in color, and contains many components other than sucrose, such as polysaccharides, colorant molecules, plant pigments and amino acids which must be removed.

Colorants in Sugar Processing
The two main sources of sugar color are (i) plant derived colorants and (ii) color formed during processing. In sugarcane processing, the major colorants tend to be plant pigments, especially

phenolics, that are associated with hemicellulosic polysaccharides found in the sugarcane plant. These colorants change little during processing [2] and would appear to be good candidates for enzymatic breakdown. In comparison, sugarbeet colorants are generally produced during processing. These colorants are mostly alkaline degradation products of fructose and glucose; and also melanoidin, colorants that are formed by the reaction of sucrose or invert with amino acids. [3] Other colorants, called melanins, are very dark colorants formed from the enzymatic reaction of amino-phenols released from sugarbeets on processing.

Sugarbeet color is generally lower than sugarcane colorant. At the same time, beet sugar can be crystallized from a much darker solution than cane sugar, highlighting the different nature of the colorant types in beet and cane processing. Therefore, the raw sugar produced from sugarcane must be further refined to remove more color to produce refined white sugar. Figure 1 shows the relative color in sugarcane and sugarbeet processing. White sugar color is typically in the range of 20 IU. As shown in Figure 1, cane sugar processing products range from over 14,000 IU for the raw juice to 1000 IU for raw sugar, and down to white sugar levels after refining. [2] The most effective color removal step for sugar is crystallization. In sugar processing, more color is transferred to the crystal in sugarcane than in sugarbeet. If enzymes can be used to reduce the color of the sugar processing products by degradation of color forming components in raw juice, less color may be transferred to the crystal.
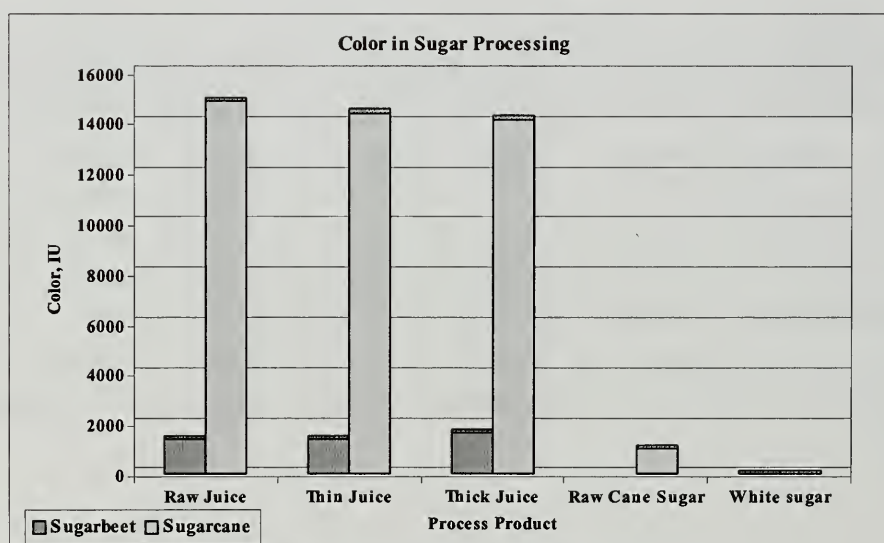
Figure 1. Color in sugar processing products.

Polysaccharides in Sugar Processing

Another potential problem in sugar processing is the presence of polysaccharides. Processing problems caused by polysaccharides can lead to delays and losses in factories and refineries. Polysaccharides decrease filterability, boiling time and recovery and can disrupt the sucrose crystal structure, resulting in elongated or other misshaped crystals, leading to problems in centrifugation, and end user products. For example, when sucrose with elongated crystal structure is used in hard candy production, the candy does not take the shape expected, leading to problems for candy producers. [4]

As with colorants, sugarcane and sugarbeets have different polysaccharides. In sugarcane, the common polysaccharides are indigenous sugarcane polysaccharide (ISP) [5] and starch. ISP is an arabinogalactan found in soluble cell wall hemicellulose. Starch is found mostly in the leaves and growing points of the sugarcane plant, and is transferred to the raw juice when the sugarcane is crushed in the mill. Starch is not a problem in sugarbeet manufacture. A very problematic polysaccharide is dextran, a microbial polysaccharide that results from infection with Leuconostoc bacteria. Dextran occurs in both beet and cane processing, but can be more prevalent in cane processing due to tropical conditions. Pectin is the main polysaccharide in sugarbeet processing. The majority of the pectin structure consists of partially methylated poly-$\alpha(1\rightarrow4)$-D-galacturonic acid residues with areas of alternating α-$(1\rightarrow2)$-L-rhamnosyl-α-$(1\rightarrow4)$-D-galacturonosyl sections containing branch-points with mostly neutral side chains of mainly L-arabinose and D-galactose. This polysaccharide can be found in a relatively high concentration of 1-2% on beets. Galactan and araban are other polysaccharides found in sugarbeets, [6] but these have not been well defined and probably represent soluble hemicellulose. The initial stages of beet juice processing are designed to eliminate pectin.

Currently the cane sugar industry employs two enzymes to aid in the processing of sugarcane juice – amylase to control starch and dextranase to control dextran. α-Amylase is used to hydrolyze and remove starch (α-$(1\rightarrow4)$-α-D-glucan) from solution. High starch content in juice can lead to many processing problems, such as slow boiling rates and lower sucrose yields. Another problem in sugar processing addressed by enzymes, when needed, is dextran (α-$(1\rightarrow6)$-α-D-glucan) that is formed when sugarcane or sugarbeet deteriorates. Dextranase is used to hydrolyze and remove dextran from process solutions. [1]

The objective of this study was to determine if certain enzyme functionalities could reduce color, turbidity and/or polysaccharides in raw cane and beet juice. Enzymes were chosen on the basis of their functionalities to attack hemicellulosic, cellulosic and phenolic moieties.

Experimental

Raw mixed cane juice was obtained from a mill in Louisiana. Sugarbeet juice was provided by a sponsor of SPRI. The experimental conditions chosen were a compromise between optimal enzyme reaction conditions and juice processing conditions. Enzymes were dosed at 500 ppm added to 100 mL juice, and the mixture allowed to react at 50° C for 30 min, with mild shaking. The native pH of the raw juice, usually in the range of 5.5 to 6.0, was used. After treatment with the enzyme, the juice was tested for color, turbidity, total polysaccharides, and sucrose degradation. Color was measured by ICUMSA Method GS1/3-7, and turbidity was determined by difference using the same method. The SPRI method for total polysaccharide was used. [7] Enzyme functionalities included cellulase, xylanase, pectinase, amylase, glucanase, glucosidase, polyphenol oxidase, hemicellulase, pullulanase, and arabanase. Table I lists the commercially available enzymes and enzyme mixtures used in this study and their functionalities.

Control samples for the cane and beet raw juice were prepared by heating 100 mL juice to 50° C for 30 min.

Table 1. Enzyme functionalities

Commercial Enzyme	Functionality
AMG 300L	Amyloglucosidase
BAN 240L	Amylase
Cellulase	Hydrolyzes cellulose
Celluclast	Cellulase and other carbohydrases
DEPOL 40L	Cellulase, xylanase, and pectinase
DEPOL 112L	Cellulase, xylanase
DEPOL 670L	Cellulase, xylanase, pectinase, ferulic acid esterase
Driselase	Cellulase, pectinase, laminarinase, xylanase, and amylase
Fermcolase	Oxidase
Finizyme	β-Glucanase
α-glucosidase	Hydrolyzes terminal, non-reducing 1,4 linked α-glucose residues
β-glucosidase	Hydrolyzes terminal, non-reducing 1.4-linked β-glucose residues
Hemicellulase	Liberates galactose from hemicellulose
Inulinase	1-β-D-fructan-fructanhydrolase
Laccase	Polyphenol oxidase
LE-R	Lysing enzyme from *Rhizoctonia solani* containing yeast glucanase activity
LE-T	Lysing enzyme from *Trichoderma harzianum*, containing cellulase, protease, and chitinase
Pectinex 3XL	Pectinase
Pectinex Ultra	Pectinase
Pullulanase	Hydrolyzes (1→6)-α-**D**-glucosidic linnkages in pullulan
Rapidase A	Pecitnase and hemicellulase
Rapidase P	Amylase and pectinase
Rapidase ExColor	Pecitanse with hemicellulase
Rapidase X-Press	Pectolytic enzyme
Sucrodex	Dextranase, amylase, cellulase, β-glucanase, and xylanase
Viscozyme 120L	"carbohydrase"
Viscozyme	Arabanase, cellulase, β-glucanase, pectinase, xylanase
Xylanase	Hydrolyzes xylan

Results and Discussion

Control Samples

Previous work at SPRI has shown that mild heating of raw juice reduced color and polysaccharide due to coagulation of colloids, which causes them to precipitate out, removing a portion of these components. Therefore, a control sample of heated sugarcane or sugarbeet juice was prepared for each set of experiments.

Enzyme Treatment of Cane Juice

Heated control cane juice samples had 12.2 to 13.2% color removal and 10.9 to 12.2% total polysaccharide removal. Heating the juice increased the turbidity 11.0 to 12.5% compared to unheated juice. The color and polysaccharide content of the heated control samples were used as the baseline measurement to compare with the enzyme treated samples.

Raw cane juice was analyzed for color, turbidity and polysaccharide. A decrease of 10% in color, turbidity or total polysaccharide, above the control, was considered significant.

Nine of the 28 enzymes tested (Table 1) removed a significant amount of color. These were: Celluclast, DEPOL 40L, DEPOL 112L, DEPOL 670L, Driselase, α-glucosidase, β-glucosidase, hemicellulase, and Viscozyme L. Hemicellulase removed the most color at 18% and Driselase removed ~12% color with the other enzymes listed falling within this range. The findings are summarized in Figure 2. The functionalities that reduced color included cellulase, xylanase, hemicellulase, and glucosidase. These activities may indicate that a bond between a colorant molecule and a polysaccharide was being broken.

Total polysaccharide analysis showed that eight enzymes removed 10% or more of the polysaccharides from the raw cane juice: DEPOL 40L, DEPOL 670L, Finizyme, Inulinase, Pectinex 3XL, Rapidase P, Viscozyme 120L and Viscozyme L. Viscozyme L removed ~16% total polysaccharide and Finizyme removed ~11% with the other enzymes falling within this range. These results are also summarized in Figure 2. Enzyme functionalities that reduced polysaccharide content in raw cane juice were cellulase, xylanase, pectinase, glucanase, hemicellulase, and xylanase.

Some enzymes tested increased the polysaccharide content of the juice, but this was discovered to be due to the use of starch or other polysaccharide material used as stabilizers for the solid enzyme preparations. These enzymes included hemicellulase, Driselase, Laccase, and xylanase. The presence of stabilizers is a problem for laboratory scale experiments, but may not affect factory applications. Besides, specifications could be made that no starch or other polysaccharide be used to stabilize the enzymes. Enzymes used on a commercial scale are usually sold in liquid form. Current enzyme applications in the sugar industry, *i.e.*, α-amylase and dextranase, are liquid formulations.
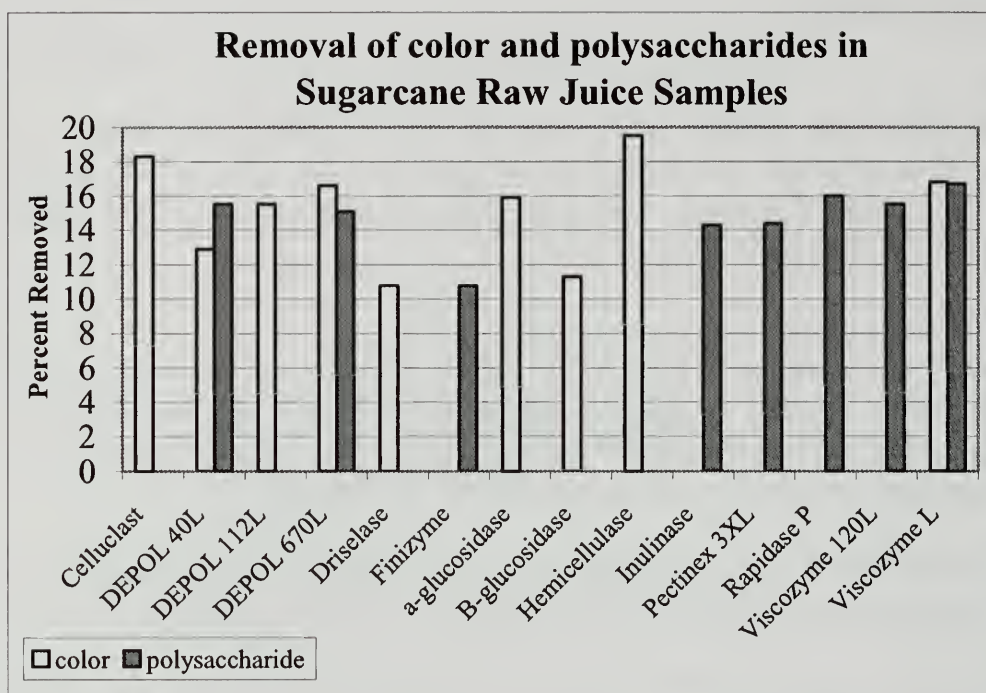
Figure 2. *Results of color and polysaccharide removal by commercial enzymes in raw sugarcane juice. Only the enzymes that removed 10% or more color and/or polysaccharidesover the control are shown.*

The effect of enzymes on juice turbidity was also determined. The heated control had increases in turbidity of 11.0% to 12.5% when compared to raw cane juice. Eighteen enzymes decreased turbidity over and above the heated control sample. LE-R decreased the turbidity by more than 10%. Three other enzymes removed significant amounts of turbidity as shown in Figure 3: cellulase (8.2%), driselase (9.1%), and LE-T (9.8%).
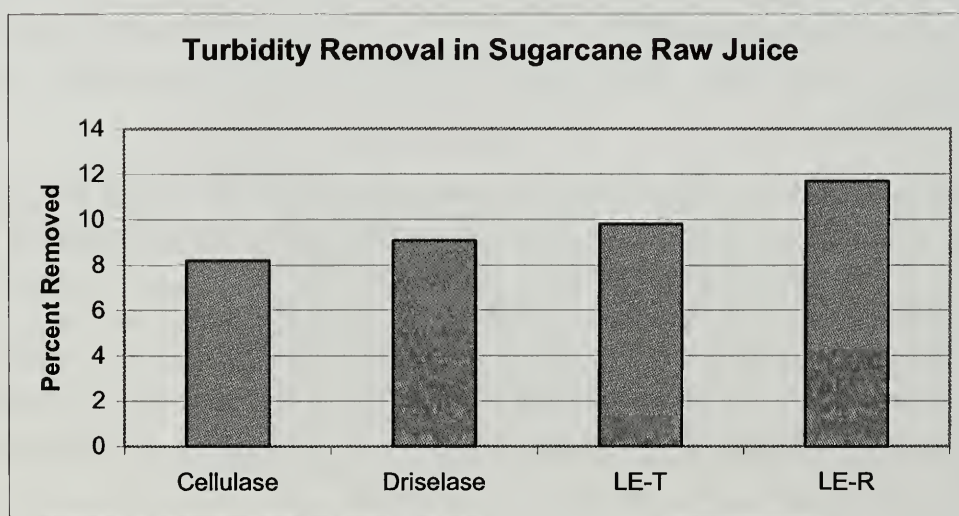
Figure 3. *Results of turbidity removal by commercial enzymes in raw sugarcane juice.*

The most important requirement for an enzyme used in sugar processing is that it not degrade sucrose. The raw juice solutions treated with enzymes that removed 10% or more color and/or polysaccharide were analyzed for sucrose degradation. The analysis was done by ion chromatography with pulsed amperometric detection, monitoring the formation of glucose and fructose. The enzymes that degraded sucrose were DEPOL 40L, DEPOL 670L, α-Glucosidase, Pectinex 3XL, and Viscozyme L. These enzymes cleave the α(1→2) glycosidic bond between glucose and fructose, destroying the sucrose molecule and, therefore, would not be suitable for use in sugar processing. Enzymes that did not degrade sucrose were cellulase, Cellclast, DEPOL 112L, Driselase, Finizyme, β-glucosidase, hemicellulase, and xylanase. These types of enzymes are potentially safe to use in sugar processing with respect to sucrose degradation.

One exception to the above observation may be Viscozyme. Viscozyme removed significant amounts of color (16.8%) and polysaccharides (16.7%) but produced glucose. Glucose hydrolysis from the terminal end of polysaccharides is a function of the Viscozyme enzyme mixture. No increase of fructose was observed in viscozyme treated samples, indicating the increase in glucose may not be from the degradation of sucrose, but rather from the hydrolysis of polysaccharides. Oligosaccharide formation was also noted in the Viscozyme treated juice.

Summary of Sugarcane Juices Treated with Enzymes

The three enzyme functionalities that significantly reduced color in the raw cane juice were cellulase, xylanase, and hemicellulase. Cellulase also decreased the polysaccharide content and turbidity of cane juice. The solid hemicellulase and xylanase increased polysaccharide content in juice due to the starch stabilizer in the enzyme formulations. Commercial mixtures of enzymes also proved useful in reducing color or polysaccharides in sugarcane juices. These mixtures included DEPOL 112L, a mixture of cellulase and xylanase, that removed 15% color and 9.5% polysaccharides. Another mixture that proved useful was DEPOL 670L. This mixture of cellulase, xylanase, pectinase, and ferulic acid esterase removed a significant amount of color and polysaccharides, but, unfortunately, degraded sucrose.

Enzyme Treatment of Beet Juice

The control sugarbeet sample was prepared the same as the sugarcane control sample. Diffusion juice (100 mL) was heated to 50° C for 30 min. Heating the diffusion juice removed 9% of total polysaccharides, increased the color by 2% and increased the turbidity by 2.6%.

As with the sugarcane samples, sugarbeet samples were tested for color, turbidity and polysaccharide removal. A decrease of 10% or more in color or total polysaccharide content was considered significant when compared to the control sample. Four of the 28 enzymes tested lowered color by 10% or more: Fermcolase (oxidase), Finizyme (β-glucanase), hemicellulase, and xylanase. Xylanase removed most color at 31.4 % and the Fermcolase removed ~17% color, with the other enzymes falling within this range. These findings are summarized in Figure 4.

Four enzymes removed 10% or more of the total polysaccharides from the raw beet juice: DEPOL 40L, DEPOL 670L, Pectinase Ultra, and Viscozyme L. Viscozyme L removed ~19.9% total polysaccharide and DEPOL 40L removed ~14.8% with the other enzymes falling within this range. These results are summarized in Figure 4.
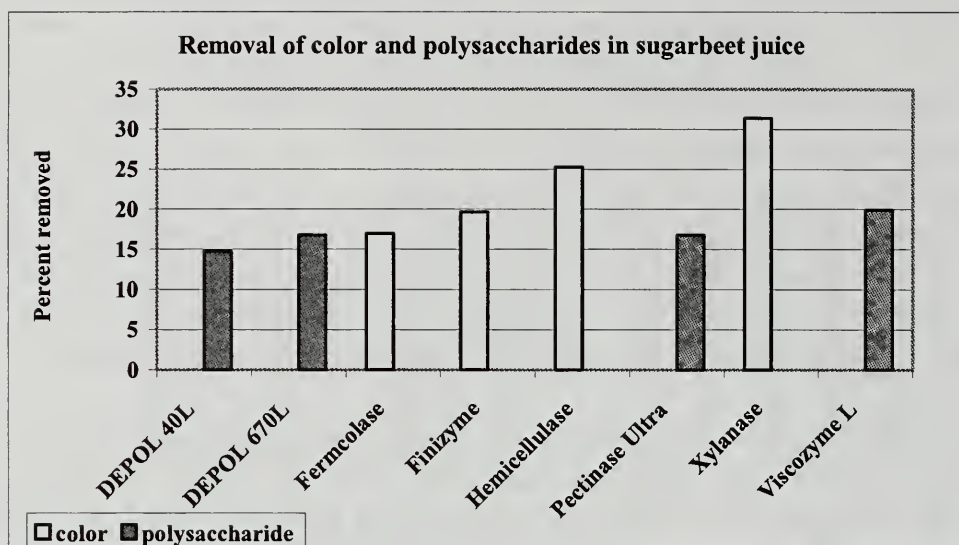
Figure 4. Results of color (light bars) and polysaccharide (dark bars) removal by commercial enzymes in raw sugarbeet juice. Only enzymes that removed 10% or more color or polysaccharide are shown.

The same observations about enzymes that increase the polysaccharide content and that degraded sucrose were made with respect to raw beet juice.

The heated sugarbeet juice control showed an increase in turbidity of 2.6% when compared to raw beet juice. None of the tested enzymes removed more than 10% of the turbidity. Several enzymes did remove moderate amounts of turbidity, as shown in Figure 5. These included Inulinase (8.5%), Pectinase Ultra (7.7%), LE-R (7.2%), and AMG 300L (6.3%).
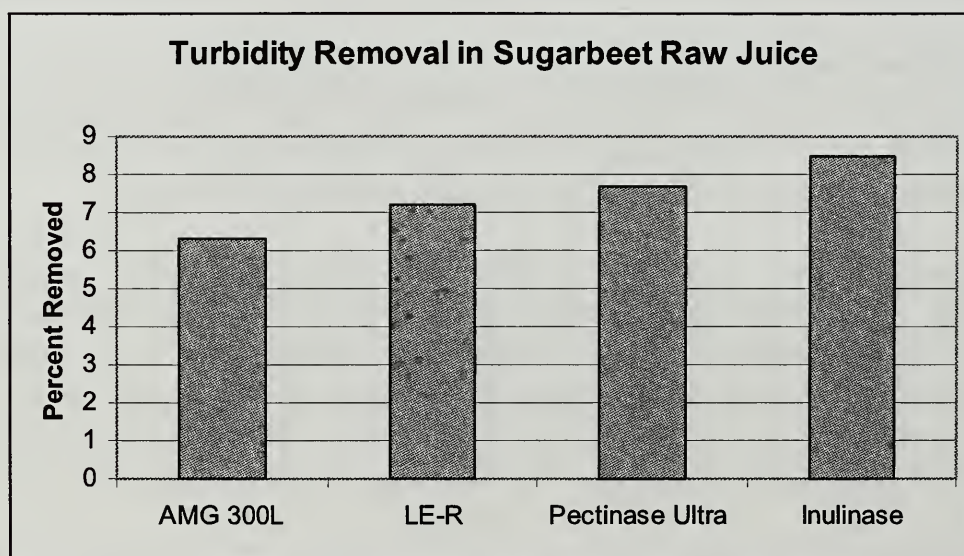
Figure 5. Results of turbidity removal by commerical enzymes in raw sugarbeet juice.

Summary of Beet Juice Results

Four enzymes significantly reduced color in the raw beet juice: Fermcolase, Finizyme, hemicellulase, and xylanase. Hemicellulase and xylanase increased polysaccharide content in juice due to polysaccharide stabilizers in the enzyme formulation. Commercial enzyme mixtures also proved useful in reducing color or polysaccharides in sugarcane juices. DEPOL 40L and DEPOL 670L are two commercial enzyme mixtures that reduced polysaccharide content in sugarbeet juice. DEPOL 40L is a mixture of cellulase, xylanase, and pectinase. Cellulase, xylanase, pectinase, and ferulic acid esterase combine to make DEPOL 670L.

Conclusion

Twenty-eight commercially available enzymes and enzyme mixtures were tested for reactivity in cane and beet raw juices for removal of color, turbidity and/or polysaccharides. The enzymes that removed a significant amount (greater than 10%) of either color or polysaccharides were further tested for sucrose degradation. It is important to note that these reactions were not conducted under optimized enzyme reaction conditions or processing conditions, but were a compromise between the two. Requirements for enzyme use in commercial sucrose processing include the following: (i) The enzyme must be commercially available, (ii) approved for food processing (GRAS - generally recognized as safe by the Food and Drug Administration), (iii) low cost, and (iv) will not degrade sucrose. Several enzyme functionalities removed colorants and polysaccharides in cane juice and beet juice. In both juice systems, several enzymes were very effective.

Processing conditions for sugarcane may be more optimal for enzyme activity because lower temperatures and pH are used in sugarcane processing compared to sugarbeet processing. Based on these preliminary studies, several enzyme mixtures are good candidates for controlling color, polysaccharide and turbidity in cane juice. For effective enzyme use in beet juice, the process may need to be modified to allow a holding time under milder conditions to allow the enzymes time to work. In either case, a holding time of about 30 minutes at 50° C was shown in these studies to allow the enzyme time to work without causing sucrose loss.

The functionality of the active enzymes also provide information on the nature of the colorant-polysaccharide interactions that take place in cane and beet raw juice systems, and indicate that hemicellulose, cellulose and xylan (components of cell walls) are implicated in both polysaccharide content and colorant content.

By removing colorants and polysaccharides from raw juice solutions prior to clarification, sugar factories can produce lower color sugar, and they may also see improvement in boiling and crystallization. The benefit of using enzymes in beet processing needs to be explored further because the majority of beet colorant is process-derived, rather than plant-derived, as in cane processing, and this study did not examine the effect of enzymes on process-derived colorants.

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Filter Clogging Material in Raw and White Cane Sugar

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Abstract

Raw and refined sugar solutions were sequentially filtered on Millipore membranes with different pore sizes, ranging from 60 μm to 0.45 μm, and the material trapped on the membrane surfaces examined by electron microscopy. The results showed that filter clogging material was caught on membranes with 1.2 μm pore size or smaller. In the case of raw sugar solutions, membranes visually appeared to be covered with a uniform coat of light brown color, whereas white sugar solutions may or may not have a small amount of color. Microscopic examination revealed an amorphous, gel-like material coating the membrane and partially embedded in it, completely covering the pores of the membrane. It is felt that this material contributes both color and turbidity during sugar processing and may arise from highly degraded bagacillo in the colloidal range. Filtration studies showed that most refined cane sugar solutions will clog a 0.45 μm pore-size membrane before a solution containing 100 g of sugar can be completely filtered. Enzyme studies showed that enzymes associated with cellulose and hemicellulose degradation improved filtration of raw sugar solutions. Hydrolysis and gas chromatographic examination of the filter-clogging material showed a polysaccharide-rich component that was high in mannose and glucose, indicating a different composition from the soluble indigenous cell wall polysaccharide of cane. A similar material was shown to coat ion exchange resin beads.

Introduction

Studies on the causes of filtration impedance in sugar processing have shown that the impeding effect is as much related to the size of the impurities as to their chemical nature. Filtration impedance is caused by fine precipitates, such as those of lime, aluminum and magnesium, by insoluble sediment, and by gums and polysaccharides, including starch and dextran (Devereux and Clarke, 1984; Hidi and McCowage, 1984). One small, interesting study showed that filtration impedance correlated most strongly with turbidity, total polysaccharides and color (Vianna, 2000).

Sugar solution color is determined after filtration on a 0.45 µm cellulose acetate membrane. Raw sugar solutions always plug the membrane before all of the prepared solution (100 g of a 30 Brix solution) is filtered. Refined sugar solutions (100 g of a 50 Brix solution) also usually plug membranes before all the solution is filtered. After filtration of raw sugar solutions, the filter membranes are usually covered with a coating of light brown color. After filtration of refined sugar solutions, the membranes sometimes have a pale coating of color, but it is usually possible to visually note that there is a fine film of material retained on the filter, even when there is no color trapped on the surface of the membrane.

We report here on the discovery of a colloidal, gel-like material from raw and refined cane sugar that is retained on membranes with pore size 1.2 µm or smaller.

Materials and Methods

Sequential Filtration of a Raw Sugar
In order to visualize the nature of the insoluble matter in raw sugar, a high pol, low color sugar from Brazil was sequentially filtered on five membranes with the following pore sizes: 60 µm, 20 µm, 8 µm , 1.2 µm and 0.45 µm. The filters were dried in air overnight and examined by electron microscope.

Microscopy
For microscopic studies, all samples were first examined using a widefield stereo light microscope to determine areas of the sample of most interest to examine using the scanning electron microscope (SEM). Selected areas of the filters or groups of resin beads were attached to SEM sample stubs that had been covered with tacky glue. To make samples electrically conductive for SEM study, stubs were vacuum coated with a thin layer of gold/palladium metal. Samples were then examined directly in the SEM and selected images recorded.

Filtration of Other Sugars.
A range of raw and refined cane sugars were also examined for the presence of membrane-coating, filter-clogging substance. Thirty Brix solutions were pre-filtered on 20 µm filters, and then filtered on 0.45 µm membranes. The 0.45 µm membranes were air dried overnight and examined with the electron microscope.

Filtration Rate of Refined Sugar
Several refined cane sugars were prepared in 100 ml of 50% (w/v) solutions (50 g sugar in 100 ml). Each solution was filtered on one 0.45 µm membrane, and the amount of volume filtered and the time before the filter clogged were noted. A set of beet white sugars was similarly examined.

Enzyme Effect on Filtration.
Two raw sugar solutions were treated with various enzymes to determine if filtration was improved. Raw sugar solutions (100 g of 15 Brix) were treated with 100 ppm enzyme at 50° C for 30 min, then filtered on a 0.45µm membrane. Enzymes examined were amylase, dextranase, xylanase, hemicellulase, cellulase and α- and β-glucosidase.

Microscopic Examination of Resin Beads

We received some ion exchange resin beads from a refinery that had been processing a high dextran, high color raw sugar, and having trouble with resin fouling. The resin fouling was much more severe than usual, although similar dextran levels usually processed without this much difficulty. Microscopy was performed on unwashed beads, and beads washed with either water or 80% ethanol. Beads were cut in order to examine the interior of the bead as well as the surface of the bead.

Isolation of Filter Clogging Material from Membrane Filters. To further characterize the filter clogging material, it was isolated from 0.45 μm membranes. 50 g of a typical raw sugar (color 1174 IU; total polysaccharides 808 ppm; dextran 454 ppm; starch 185 ppm), in a 25-30 Brix solution, was pre-filtered on a 20μm filter and then filtered through 0.45 μm membranes. The solution was allowed to filter until the filter stopped draining, and another filter was then put in place. Filtration of the raw sugar took 14 filters to complete filtration. The 14 filters were air dried.

For white sugar, 100 g of refined sugar in a 25-30 Brix solution was filtered through 0.45 μm membranes. Pre-filtration on 20 μm membranes was not done. Three filters were required to filter all of the refined sugar. The filters were air dried.

To extract the membrane clogging material, the filters were cut up and soaked with 2N trifluoroacetic acid (TFA) for several hours, then drained through glass fiber and washed, for a total of 20 ml. Some color remained on the raw sugar filters after extraction. The samples were held in TFA at 100° C for two hours to allow hydrolysis of the sugars. After cooling, 1 ml was taken for total carbohydrate determination by the phenol-sulfuric acid test. The sugars in the hydrolyzed extract were converted to alditol acetates for determination by gas chromatography, using myo-inositol as the internal standard.

Results

Examination of Sediment in Raw Sugar - Sequential Filtration

The properties of the sequentially filtered sugar were: Color 524 IU; turbidity 423 IU (18.4 NTU); total polysaccharides 346 ppm; starch 127 ppm; dextran 144 ppm; sediment 210 ppm. The total amount of material retained on each membrane is shown in Table 1.

Table 1. Amount of material retained on each membrane in sequential filtration of raw sugar.

Membrane pore size, μm	ppm retained on membrane	Cumulative total, ppm
60	120	120
20	50	170
8	106	276
1.2	278	554
0.45	260	814

The ICUMSA sediment test, which measures sediment retained on an 8μm membrane, averaged 210 ppm ± 10.4 ppm, n=3. This value is close to the cumulative 276 ppm retained on the 60, 20 and 8 μm membranes. An additional 538 ppm was retained on the 1.2 and 0.45 μm membranes.

60 μm Membrane. Visual appearance of membrane: Visible drifts of pale straw colored short fibers, with appearance of bagacillo; black specks and some silvery/shiny particles, like sand or glass. The membrane had not retained any coloring matter.

Microscopy. Much of the material was fibrous with cellular structure, indicating it was plant-derived, and probably mostly bagacillo. Although a few particles were as large as 1-2 mm, most of the material on this membrane was in the range of 100-200 μm. A few particles were interpreted as sand or soil. In the 300 X magnification, plate-like shards were noted that had very fine pores throughout, probably from cane leaves. (Figures 1 and 2)

20 μm Membrane. Visual appearance of membrane: Very small drifts of material that looked the same as the 60 μm membrane, but which were much smaller and harder to see with the unaided eye. No color was retained on the membrane.

Microscopy. The particles appeared to be similar to those caught on the 60 μm membrane, but smaller in size -- fibrous pieces of plant material, small smooth sand grains, agglomerated soil particles, and smooth, plate-like shards with tiny pores. There were more soil particles in this sample than in the 60 μm membrane. (Figure 3)

8 μm Membrane. Visual appearance of membrane: Membrane was coated with a very pale straw to beige color, and there appeared to be particles on the surface which could not be distinguished by the eye, but which gave the appearance and sensation of a slight roughness.

Microscopy. The membrane surface was visually colored, but it was not obvious from microscopy where the coloring matter came from. The 100 X and 300 X micrographs showed that the platelike shards described above were distributed over the surface of the membrane. The two 500 X micrographs confirmed that these particles were similar to the ones on the 60 μm and 20 μm membranes, only smaller. They had small, fine pores and, in some cases, a fibrous structure. Cellular structure was not visible. The 1000 X micrograph showed a small amount of filamentous material. It was interpreted that these very small shard-like materials, which may be finely broken particles of bagacillo, were responsible for the over-all light brown color of the membrane and the slightly rough appearance seen by the unaided eye. (Figures 4 and 5)

1.2 μm Membrane. Visual appearance of membrane: The surface of the membrane was uniformly covered with light brown color (darker than the 8 μm membrane color described above). There was no appearance of roughness to the eye, rather a smooth appearance.

Microscopy. An isolated agglomerated clump was examined at 100 X, 500 X and 1000 X and noted to be very fine fibrous material, again probably very finely comminuted bagacillo. There was very little of this particulate material, and it could not account for the color on the membrane. However, at 1000 X, a coating phenomenon became visible, and was further elucidated at 2000 X

and 3000 X. Small portions of the membrane surface appeared to be coated with a gummy, filamentous substance. With further magnification, it could be seen that this gummy material, which appeared as interconnecting filaments or strands, had entered into the pore structure of the membrane and was present throughout the membrane. (Figures 6, 7 and 8)

0.45 μm Membrane. Visual appearance of membrane: The membrane was uniformly covered with a light brown color, similar to the 1.2 μm membrane, but lighter in color.

Microscopy. The 300 X micrograph showed what appeared like patches or blotches rather material evenly scattered over the surface. The 500 X micrograph showed these blotches to have the appearance of blobs or globs of gelatinous material. The 1000 X micrographs confirmed the gelatinous appearance of the blotches, and some appeared partially sunken into the surface of the membrane. The 3000 X and 5000 X magnifications showed that this material was plastered on the surface of membrane. It did not have the stringy, filamentous appearance of the 1.2 μm membrane, but did have the same gelatinous, coating appearance. It was interpreted that this material, a combination of colloidal polysaccharide and colorant, was responsible for the brown color on the membrane. (Figures 9 and 10)

Enzyme Effect on Filtration

Enzyme studies showed that enzymes associated with cellulose and hemicellulose degradation improved filtration of raw sugar, as did amylase and dextranase. Results are shown in Table 2.

Table 2. Enzyme effect on filtration of two raw sugars (results averaged)

Enzyme	% Improvement in filtration
Amylase	10
Dextranase	14
Hemicellulase	14
Cellulase	14
Xylanase	19
α-Glucosidase	No improvement
β-Glucosidase	No improvement

Micrographs of Membranes with Raw Sugar Filtration
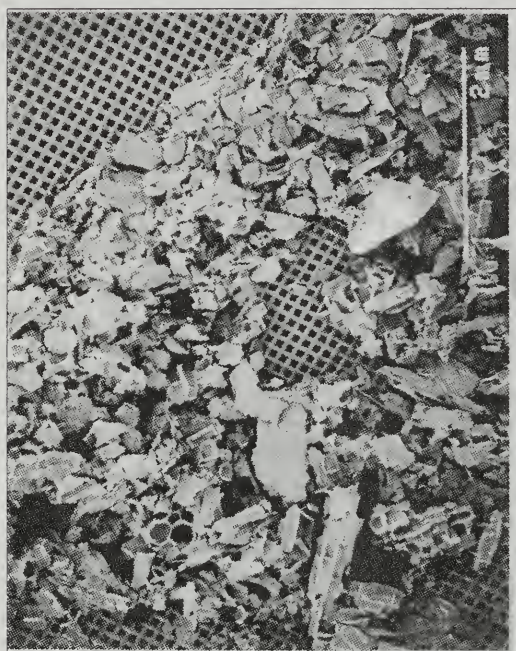
Figure 1. 60 μm membrane. Mostly plant material and a few soil particles. Taken at 20X. Mark = 2 mm.
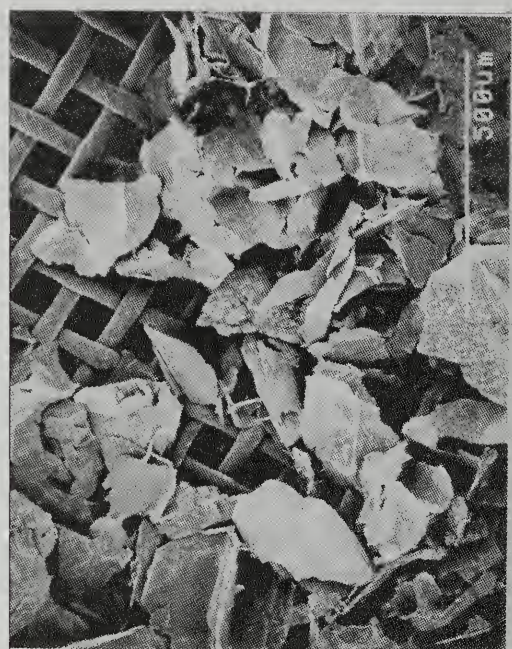
Figure 2. 60 μm membrane. Details of plant shards. Taken at 100X. Mark = 500 μm.

Figure 3. 20 μm membrane. Variety of plant material caught on membrane. Taken at 300X. Mark = 100 μm.
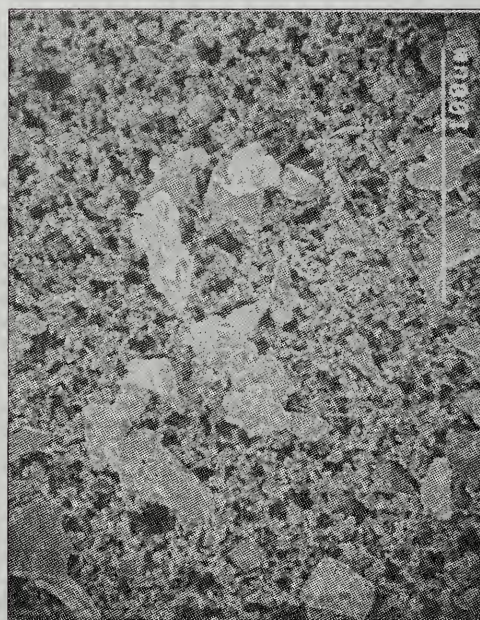
Figure 4. 8 μm membrane. Fine shards and fibrous material responsible for color on membrane. Taken at 500X. Mark = 100 μm.
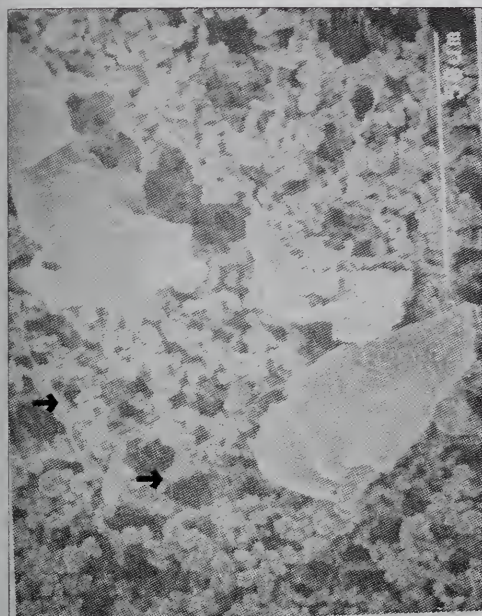
Figure 5. 8 μm membrane. Details of shards and some gummy filamentous material in lower center-left(➜). Taken at 1000X. Mark = 50 μm.
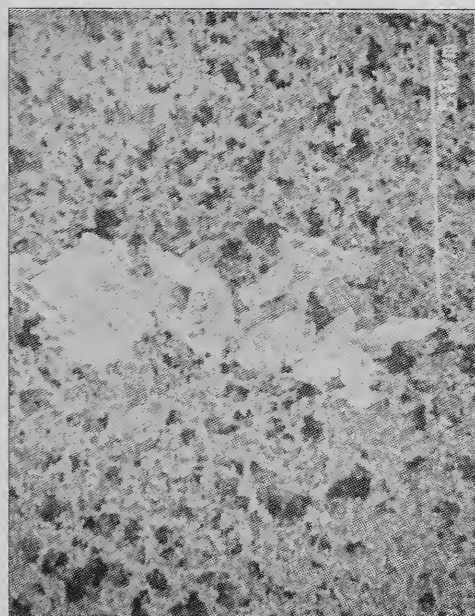
Figure 6. 1.2 μm membrane. Shows an isolated, agglomerated clump of fibrous plant material. Taken at 1000X. Mark = 50 μm
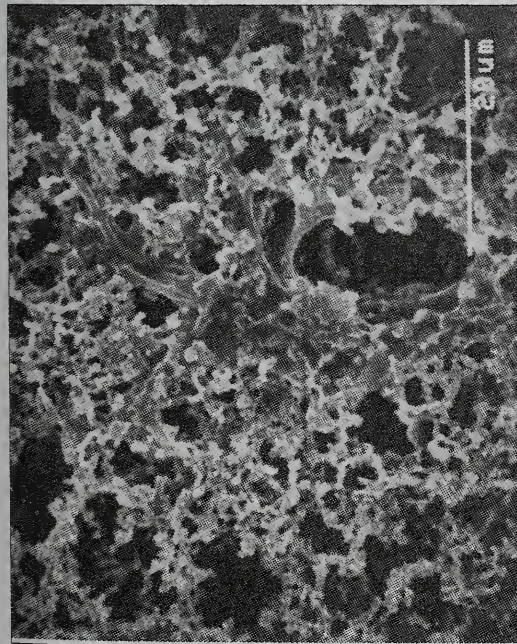
Figure 7. 1.2 μm membrane, 2000 X. Coating of membrane below surface by gummy filaments is visible, upper middle. Taken at 2000X. Mark = 20 μm.
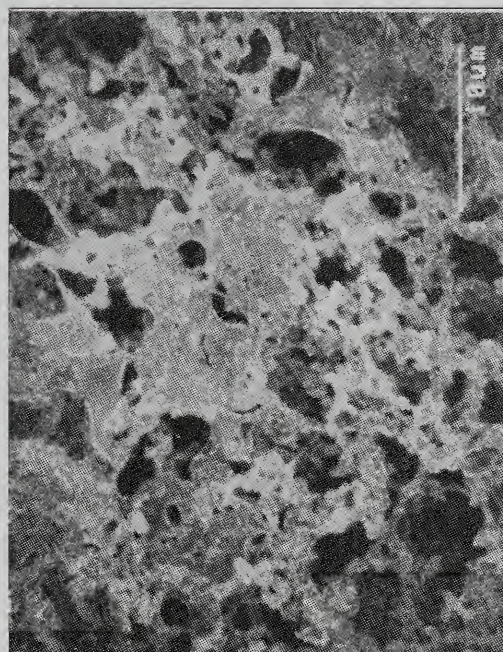
Figure 8. 1.2 μm membrane, 3000 X. Coating of membrane surface with gummy material. Taken at 3000X. Mark = 10 μm.
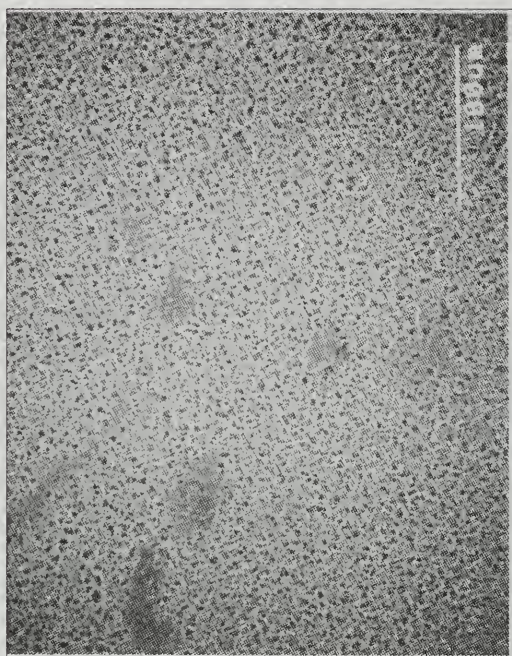
Figure 9. 0.45 μm membrane. Patches and blobs on surface of membrane, responsible for color of membrane. Taken at 300X. Mark = 100 μm.
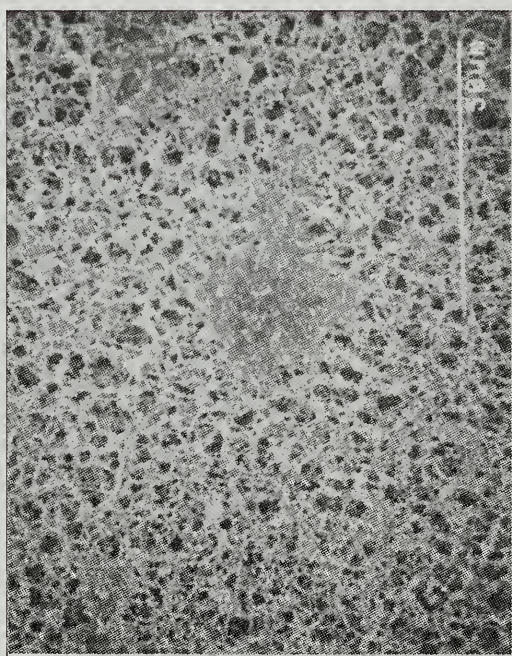
Figure 10. 0.45 μm membrane. Shows detail of one of the patches of colloidal material partially embedded in the membrane surface. Taken at 1000X. Mark = 50 μm.

Filtration Rate of White Sugars

Filtration of nine cane refined sugars and six beet white sugars showed the contrast in filterability of the two types of sugar on a 0.45 μm membrane.

Table 3. Filtration of White Sugars (100 g at 50 Bx on 0.45 μm membrane)

Refined Cane Sugar	Filtration time, min*	Percent (%) filtered on one membrane
C&H Bakers Special	8	81.5
C&H Con Sanding	8	79.0
Dixie Crystals	8	73.3
Canada	8	71.0
Canada	8	63.6
South Africa, floccing	6	58.0
Colonial, non-floccing	8	55.6
Colombia floccing	10	37.5
Venezuela floccing	5	26.9
Beet White Sugar	**Filtration time, min**	**Percent (%) filtered on one membrane**
U.S.	5	100
Spain	6	100
British Sugar	10	100
U.S.	5	100
U.S.	5	100
U.S.	5	100

*Filtration time is the time at which either the entire solution had filtered through the filter or the time at which the solution stopped filtering.

Membrane-Coating Material in Raw and White Sugars

Microscopic examination of several raw, refined and Indian plantation sugars all showed the presence of membrane-coating material. All sugars were pre-filtered on 20 µm membranes prior to filtration on 0.45µm membranes. Figure 11 shows two raw sugars, and Figure 12 shows two refined sugars.
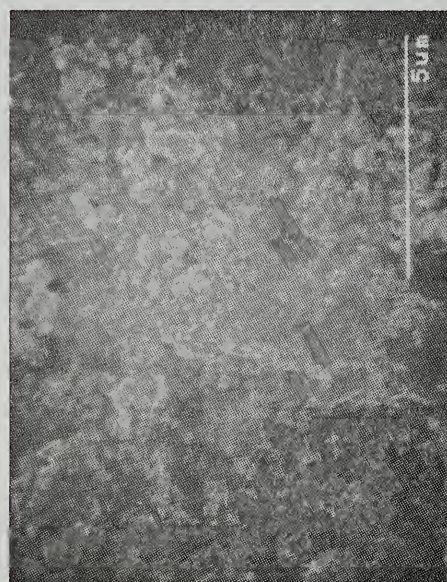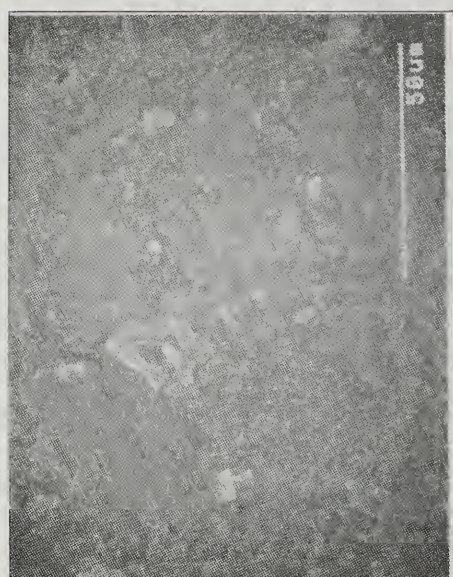
Figure 11. Micrographs of raw sugar filtered on 0.45 µm membranes. Left: Guatemala raw sugar, taken at 1000X; mark = 50 µm. Right: Louisiana raw sugar, take at 10,000X; mark = 5 µm.
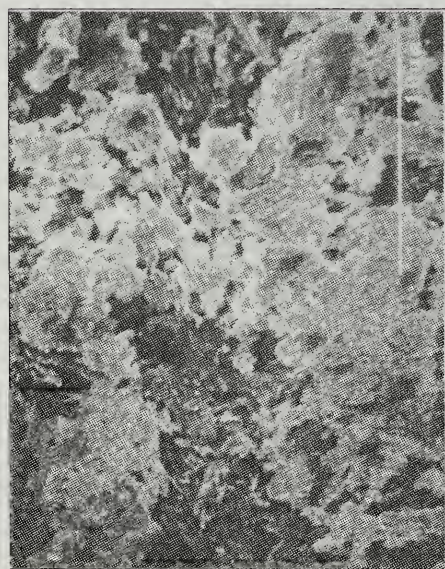
Figure 12. Micrographs of refined cane sugar filtered on 0.45 µm membranes. Left: South African refined sugar, taken at 5000X; mark = 10 µm. Right: C&H Bakers Special, taken at 5000X; mark = 10 µm.

Examination of Fouled Resin Beads

We came across an instance of the importance of the colloidal polysaccharide-color complex/gel material in refining. A refinery that was processing a high-dextran, high color raw sugar observed that long before resin exhaustion was expected, the resin had stopped decolorizing the syrup. Regeneration of the resin required extra caustic and acid treatment. Microscopy (Figures 13-18) showed that the resin beads were completely covered with material that looked very much like the raw sugar colloidal gel that coats 0.45 μm membrane filters. It was highly colored (the spent beads were black), and the color penetrated about 100 microns into the interior of the resin bead. The unused beads (Figures 12 and 17) were smooth and clean in appearance, and on 4000 X magnification, showed a surface covered with small pores (Figure 17).

The unwashed, spent resin was completely covered with a variety of lumpy material, appearing mostly gel-like (Figure 14). When washed with water, the beads still retained a complete, gel-like coating of material (Figure 15). Washing with alcohol did not remove the coating material (Figure 16). Figure 18 shows a 4000 X magnification of the material on a spent resin bead, which can be compared to the clean, unused resin bead, at the same magnification, in Figure 17.
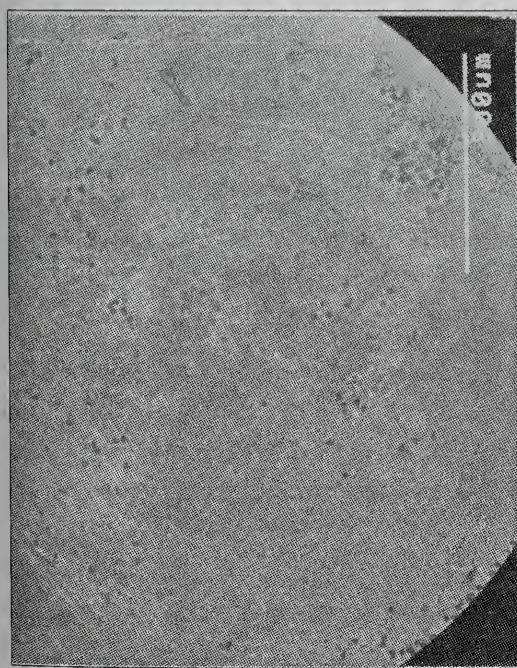
Figure 13. Clean, new resin bead. Taken at 200X. Mark = 200 μm.
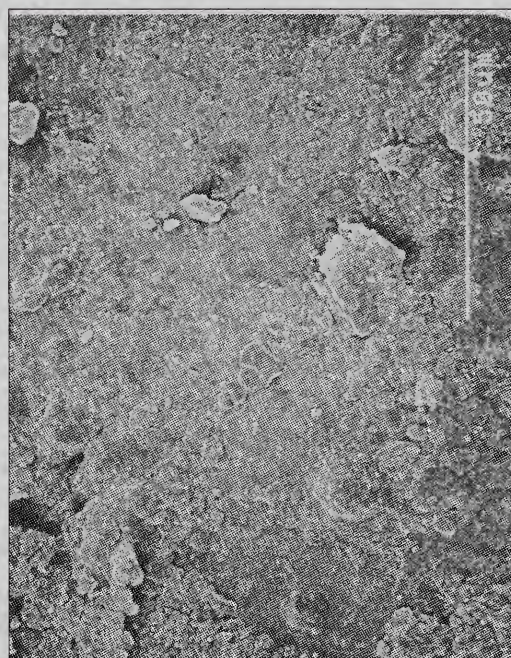
Figure 14. Surface of spent, unwashed resin bead. Taken at 1000X. Mark = 50 μm.

Figure 15. Surface of a water-washed spent resin bead. Taken at 200X. Note that material still coats the surface of the resin. Mark = 50 μm
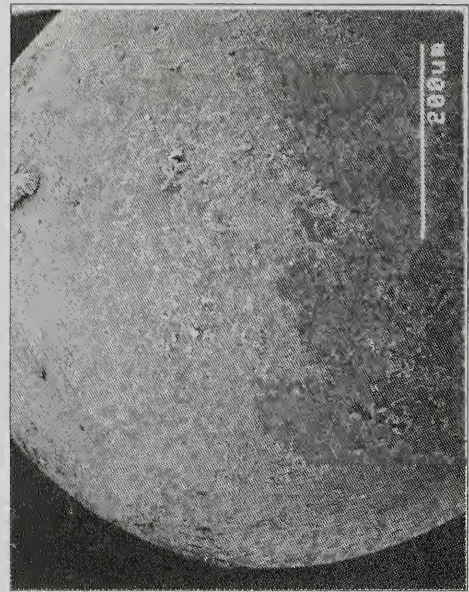
Figure 16. Surface of alcohol-washed spent resin bead. Taken at 200X. Note that material still coats the surface of the resin. Mark = 200 μm.

Figure 17. Surface of a clean, unused resin bead, showing pores. Taken at 4000X. Mark = 10 μm.

Figure 18. Surface of a spent resin bead. Pores are completely covered. Taken at 4000X. Mark = 10 μm.

Composition of the Material Coating the Filters

Filtration of the raw sugar took fourteen 0.45 μm pore size filters to completely filter the 50 g of raw sugar. It took three filters for 100 g of white sugar. The phenol sulfuric acid test showed that approximately 24% of the isolated raw sugar material and 40.5% of the white sugar material was carbohydrate. Table 4 shows the proportion of sugars present in the hydrolyzed isolate. The material consisted mostly of glucose, and was also high in mannose; galactose was absent. This is a different composition from soluble cane indigenous sugarcane polysaccharide (ISP), which is high in arabinose and galactose, with mannose present at around 1%.

Table 4. Composition of filter-clogging material in raw and refined cane sugar.

Sugar	Raw Sugar	White Sugar
Rhamnose	0.94	0.78
Arabinose	0.99	1.29
Xylose	0.53	0.71
Mannose	6.32	9.09
Glucose	91.22	88.13

Discussion

Sequential filtration of raw sugar on increasingly smaller pore sized membranes revealed the presence of a colloidal component not previously identified.

The material caught on the 60 μm and 20 μm membranes was the expected assortment of plant material, sand and dirt, with an occasional fiber. These two filter sizes did not retain any coloring material. The material caught on the 8 μm membrane appeared to be very fine bits of partially degraded plant material that gave the membrane surface a uniform brown color. Of note was the material caught on the 1.2 μm and 0.45 μm membranes. Both membranes retained color. Microscopic examination revealed a gel-like or gummy material that coated portions of the membrane. The material often appeared to be partially embedded in the membrane, as if part of it was able to penetrate into the pores. This material blocks filters and contributes color in processing. It may form part of an insoluble, colloidal colorant-polysaccharide complex.

The ICUMSA sediment test, which measures sediment retained on an 8μm membrane averaged 210 ppm ±10.4 ppm for this sugar. This value was close to the cumulative 276 ppm retained on the 60, 20 and 8 μm membranes (Table 1). An additional total of 538 ppm was retained on the 1.2 and 0.45 μm membranes.

Definition of a colloid. Particles less than 1.2 μm in size fall into the category of a colloid, which is defined as a stable system of small particles, typically 0.001 micron to 1 micron in any dimension., dispersed within a continuous medium in a manner that prevents them from being filtered easily or settled rapidly.

The same sort of material was noted in a range of representative raw, refined and plantation white sugars, suggesting that this is likely to be a characteristic component of cane sugar. Filtration studies showed that refined sugar filtration is impeded by this colloidal material (Table 3). All refined sugars tested showed some degree of filtration impedance, with only 81.5% of the total filtering through for the best sugar and as little as 27% filtering before clogging membranes. Although there appears to be a correlation with impeded filtration and the tendency to form acid beverage floc (Table 3), the sample size was too low to reach this conclusion. In contrast, beet sugars show a tendency to have less filtration impeding material (Table 3).

Colloidal material is not measured in the sediment test, and there is no established test for this material. The quantity of colloidal and pre-colloidal / colloid-like matter can be quite high, as shown by the 538 ppm present in a high pol, low color Brazilian sugar (Table 1). We here define pre-colloidal or colloid-like material as that which was caught on a 1.2 μm membranes after filtration through an 8 μm membrane (representing material that is in the range of 9-1.2 μm).

An examination of the amount of colloidal and pre-colloidal/colloid-like material in five representative white sugars is shown in Table 5, along with the sediment in each sugar. It is noted that the amount of this material is significant, in the range of 29 to 111 ppm.

Table 5. Sediment and colloidal material in refined cane sugar (ppm). Values are corrected for sucrose remaining on the membrane

Refined Sugar*	Sediment	1.2 μm	0.45 μm	Total colloid-like, ppm
#271	68	37	74	111
#345	18	22	32	64
#640	42	6	23	29
#643	24	11	29	40
#784	46	32	32	64

* Each sugar is from a different refinery: two from the U.S., one from Canada; two from Europe.

Compositional studies revealed that at least 24-40% of the colloidal type matter was carbohydrate (polysaccharide), but our TFA extraction procedure may not have been sufficient to extract and solubilize all the material off the membrane, or, alternatively, to hydrolyze it, especially if part of it is cellulosic in nature. Part of the composition may also be lignin in nature.

Godshall (2004) recently discussed "hidden color" (unmeasured color) in raw suga, defined as color that remains on 0.45μm filters, which is not measured by the color test, nor picked up by the sediment test. The colloidal material described in this report may explain the nature of the unmeasured/hidden color in cane sugar. The results of the enzyme/filtration studies and the composition of the sugars suggests that this material is hemicellulosic and/or cellulosic in nature. We believe that it arises from bagacillo that has been partially degraded and pulped in the clarifier due to high pH, temperature and agitation, until it is pre-colloidal to colloidal in size. Cuddihy (2006) recently referred to the "tea brewing effect" that the clarifier has on bagacillo, which he felt added color to cane juice. Les Edye (personal communication) refers to this material as cellulosans, or semi-soluble cellulose fragments.

Conclusion

The discovery in raw sugar of colloidal material that plugs filters is reported. Microscopy showed that the material is in the colloidal size range, and can be present in a significant concentration. This material can carry through the process to the refined sugar and cause filtration impedance in white sugar. This colloidal, gel-like material can coat and blind resin beads used in the refining of raw sugar. Enzymatic studies showed that filtration was improved in slow-filtering raw sugars after treatment with several enzymes, including amylase, dextranase, cellulase, xylanase and hemicellulase. It is already known that dextran and starch will impede filtration, and the results with amylase and dextranase confirmed that these will slow filtration in raw sugar and can be removed by enzymes, improving filtration performance. These results indicate that the portion of the filter-plugging material that is not composed of starch and dextran is likely to be hemicellulose and degraded cellulose that arises from small bagasse particles (known as bagacillo) that are entrained in the cane juice and undergo degradation during clarification. Bagasse is composed of cellulose, xylan and hemicellulose. During sugarcane processing, the bagasse is finely chopped up and undergoes mechanical and heat damage and pH changes, which cause some of the bagasse to degrade into an insoluble colloidal material that contains both color and polysaccharides in its structure.

Summary

In summary, the following findings are reported:

1. Filter-clogging material in cane sugar (both raw and refined) is less than 8 μm in size.
2. Filter-clogging material is colloidal and pre-colloidal or colloid-like (0.45-8.0 μm in size).
3. The amount of filter-clogging material is significant in raw sugar (at least 300 ppm).
4. Filter-clogging material in refined sugar is also significant, in the range of 30-100 ppm.
5. The material is gummy or amorphous; it is not present in discrete particles.
6. It can blind filters and foul resin beads.
7. It contributes color in processing.
8. At least 40% of the material is carbohydrate/polysaccharide.
9. Enzyme and composition studies indicate it is partly cellulosic and partly hemicellulosic.
10. It may be a highly degraded form of bagacillo.
11. It may form part of an insoluble, colloidal-like colorant polysaccharide complex.
12. This material represents "hidden color" and "turbidity". (Hidden color = Color not measured by the ICUMSA test using 0.45 μm membrane filters.)

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Mannitol as a Sensitive Indicator of Sugarcane Deterioration and Bacterial Contamination in Fuel Alcohol Production

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Abstract

Mannitol, formed mainly by *Leuconostoc mesenteroides* bacteria, is a very sensitive indicator of sugarcane deterioration that can predict processing problems. A rapid (4 to 7 min) enzymatic method has been developed to measure mannitol in juice pressed from consignments of sugarcane delivered to the factory. This screening tool will allow factory staff to know rapidly which consignments of cane will affect processing negatively or reject consignments that will cause unacceptable processing problems. This method can be easily performed using existing equipment in sugarcane factories, with mannitol being measured spectrophotometrically using mannitol dehydrogenase (MDH) as the enzyme catalyst. The stability of the reagents, limited cane juice preparation, linearity, accuracy, and precision are described. The method is highly specific for mannitol and is not affected by the presence of sucrose, glucose, fructose, or dextran. The current cost is only ~60 U.S. cents per analysis. Mannitol has also been proven to be an advantageous indicator of bacterial contamination - one of the main factors causing drops in fuel alcohol fermentation yields as well as yeast (Saccharomyces) flocculation and foaming problems. Compared to other indicators, mannitol is not produced by yeast cells but only by some contaminating bacteria (mostly Lactobacillus strains) during fermentation. Its presence can account for unexpected yield drops, and it can be measured easily. A strong correlation existed between mannitol formation and bacteria counts in sugarcane juice and molasses fermentations with induced mannitol producing bacterial contaminations.

This paper won the Margaret A. Clarke Best Paper Award

Introduction

The delivery of consignments of deteriorated sugarcane to factories can detrimentally affect multiple process units and even lead to a factory shut-down. Currently, there is no reliable, rapid, easy and inexpensive method to measure cane deterioration at the factory. This has meant that factory staff have not been able to screen individual consignments of cane and, thus, are unable to know which consignments would detrimentally affect processing in order to reject unsuitable consignments of cane.

The major (but not sole) contributor to cane deterioration in the United States is Leuconostoc, lactic acid bacterial infection. Dextran is considered the major deterioration product from Leuconostoc infection. Current methods to determine dextran suffer from being either too long and complicated, not specific enough, too expensive (Rauh, *et al.*, 2001), or too difficult in the interpretation of results (Clarke, *et al.*, 1987).

Numerous metabolic products other than dextran are formed by Leuconostoc which are of importance in sugar manufacture, including levan (a fructose polysaccharide) and mannitol. Mannitol is a sugar alcohol that does not degrade under processing conditions (Eggleston, *et al.*, 2004), can be contained in large amounts in factory syrups and massecuites processed from deteriorated cane, and directly affects processing by reducing sugar recovery (Bliss, 1975) and evaporation rates (Eggleston, *et al.*, 2007). Mannitol has been repeatedly proven to be a sensitive measure of sugarcane (Eggleston, 2002, Eggleston and Legendre, 2003, Eggleston, *et al.*, 2004) and sugarbeet deterioration (Steinmetz, *et al.*, 1998; Thielecke, 2002). Recently, Eggleston, *et al.*, (2007) found that approximately ≥ 2500 ppm/Brix of mannitol in sugarcane juice predicted processing problems at the sugarcane factory.

Previously in the sugar industry (Steinmetz, *et al.*, 1998; Eggleston, *et al.*, 2004), mannitol has been measured with IC-IPAD (ion chromatography with integrated pulsed amperometric detection) – a sophisticated technique that cannot be used at the industrial site because of its expense, complication, and the level of expertise required of the operator. Consequently, an enzymatic method to measure mannitol in sugarcane juices at the factory was developed. An enzymatic assay was chosen for its high specificity and because a chemical method would be too complicated, require unsafe chemicals and temperatures, and take too long.

Mannitol is currently determined in hospitals as an indicator of intestinal permeability, with mannitol being measured enzymatically in urine (Hessels, *et al.*, 2003. Mannitol dehydrogenase (MDH) is used to convert mannitol to fructose in the presence of co-enzyme NAD^+. The NADH formed can be easily measured spectrophotometrically at 340 nm:

$$\text{Mannitol} + NAD^+ \xrightarrow{\text{Mannitol Dehydrogenase}} \text{Fructose} + NADH + H^+$$

By extrapolating Eggleston's research on the use of mannitol to predict sugarcane deterioration

(Eggleston, *et al.*, 2004) to the fuel alcohol industry, it was recently discovered that mannitol is an advantageous indicator of bacterial contamination in fuel alcohol production from sugarcane juice or molasses (Basso, 2005). Commercial fuel ethanol in Brazil is currently produced by a fed-batch or continuous fermentation process of sugarcane juice and/or molasses by *Saccharomyces cerevisiae* culture yeast with cell recycle. Microbial contaminants (including bacteria and wild yeast) can also be recycled with the culture yeast that can cause many problems due to competition between bacteria and yeasts for the same substrate. Bacterial contamination of alcohol fermentations is regarded as a major technological problem that can cause (i) significant drops in fermentation yields, (ii) yeast (Saccharomyces) flocculation (not all contaminating bacteria cause floc formation), (iii) foaming problems (see Figure 1), and (iv) production of by-products that detrimentally affect the quality of distillates. Bacterial contamination control in Brazil is currently undertaken in (1) factory crushing by cleaning with hot water or the sometime addition of biocides (depends on the factory) or in (2) fermentation by sulfuric acid washing of yeast cell suspensions (Simpson and Hammond, 1989) or the sometime addition of antibiotics (penicillin, virginiamicin, Kamoran HJ) (Oliva-Neto and Yokoya, 2001). Oliveira, *et al.*, (1993) developed a procedure to optimize the use of antibiotics by evaluating the sensitivity of the bacterial population. However, bacterial contaminants are frequently adaptable to the products used for their control, particularly antibiotics, which makes industrial control difficult.
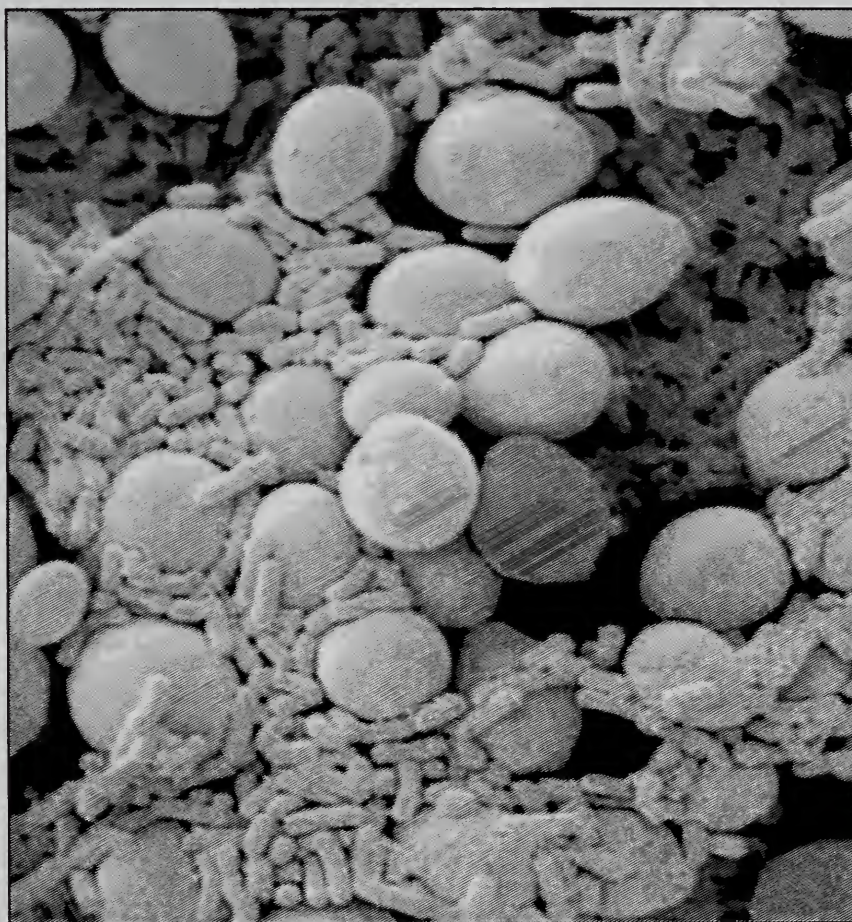
Figure 1. Bacterial contamination of fuel alcohol producing yeast. Photograph reproduced with permission from Fermentec Ltda, Brazil.

To effectively control bacterial contamination, monitoring and measurement are essential. Typical monitoring methods are either direct or indirect. Direct methods include the counting of live

bacteria by light microscopy (15 min), or plating of bacteria on rich medium (very time consuming). Indirect methods include the acidification of the culture medium, or the measurement of lactic acid. However, some methods are not specific enough for bacterial contamination, while others could be tedious, complex, or expensive.

In this paper we describe the development of a rapid, enzymatic method using MDH that can be undertaken at the factory to measure mannitol in pressed cane juices, which represent individual sugarcane consignments. We also describe the use and advantages of using mannitol as an indicator of bacterial contamination in fuel alcohol production.

Materials and Methods

Development of Enzymatic Method to Measure Mannitol

Enzyme, Chemicals, Sugarcane Juices and Buffers
Mannitol dehydrogenase (EC 1.1.1.67) was purchased as a freeze-dried powder (8.45 U/mg dry weight) from Biocatalysts Ltd, Cardiff, Wales. All chemicals were analytical grade. Dextran T2000[TM] (MW ≥ 2,000,000 Da) was from G. E. Healthcare (U.S.). Sugarcane juices were obtained from Louisiana factories. All juices (120 ml) were stored with 5 drops of biocide (Bussan 881[TM], Buckman Labs.) in a -60 °C freezer. Glycine buffer (100 mM; pH 10.5) and phosphate buffer (25 mM; pH 6.0) with 30 % glycerol were prepared (Eggleston and Harper, 2005).

NAD solution
NAD (0.22 g) was dissolved in 10 ml of de-ionized water and prepared *daily*.

Preparation of Enzyme
A stock solution of enzyme was first prepared by dissolving 0.01 g freeze-dried MDH in 1 ml ice cold phosphate + 30 % glycerol buffer. For the assays, a further dilution was made by pipetting 100 μl of stock/10 ml phosphate + 30% glycerol buffer (10,000-fold dilution). Both the stock and diluted enzyme solutions were stored in a -20 °C freezer. The stock solution can be stored for ~1 month, and the diluted enzyme for 1-2 weeks.

Mannitol Dehydrogenase Activity
See Eggleston and Harper (2005) for method.

Factory Mannitol Enzymatic Method
The method was first standardized using five mannitol standards (1, 10, 100, 500 and 1000 ppm) diluted in de-ionized water, to generate a linear standard curve and equation. A new standard curve must be generated for each batch of enzyme. Sugarcane juice was first diluted 1:1 (i.e., 2-fold) in glycine buffer and then filtered through a 0.45 μm then 0.22 μm pore-size PVDF filter. For difficult to filter samples, celite can be first added to the juice before filtering through the PVDF filters or the juice can first be filtered through Whatman[TM] 91 filter paper (185 mm; 10 μm). (NOTE: If PVDF filters are not available then add 0.5 g celite to 10 ml juice in a syringe body, mix, filter juice through a glass filter, discard first 2 ml of filtrate, and dilute 2-fold with glycine buffer). To two test-tubes the following are added:

Sample Test-Tube	Blank Test-Tube
1.4 ml glycine buffer	1.4 ml glycine buffer
0.2 ml diluted and filtered juice	0.2 ml diluted and filtered juice
0.2 ml NAD	0.2 ml NAD
0.2 ml MDH enzyme	0.2 ml water

The test-tube mixtures were vortex stirred and immediately added to a 1 cm cuvette, then placed in a spectrophotometer. The change in absorbance at 340 nm is measured from 0 to 5 min. Final absorbance was [sample absorbance - blank absorbance]. Calculations were based on the equation of the standard curve and dilution factors. For deteriorated juices containing high amounts of mannitol which cause the mannitol absorbance to be higher than the upper limit of the standard curve, further dilutions of 1:3 (4-fold) or 1:7 (8-fold) in glycine buffer are required.

Effect of Temperature on the Factory Enzymatic Method
The factory mannitol method was followed except 0.2 ml of mannitol (1400 ppm) replaced the juice and, after the enzyme was added, the two test-tubes were placed in a shaking (90 rpm) waterbath (Julaba SW22) at different temperatures (23-48° C) for 5 min, before the absorbance was measured.

Effect of Added Sugars on the Factory Enzymatic Method
Model solutions were made to simulate levels of sugars in a typical sugarcane juice, and all the solutions had a final °Brix of 14.0. All the solutions contained either 1000 or 2000 ppm mannitol. Glucose and fructose additions were on ~3% on solids basis, sucrose ~90% solids. Dextran was added at 1000 ppm level. The factory mannitol method was followed except the model sugar solution containing mannitol (1000 or 2000 ppm) replaced the juice.

Haze Dextran in Sugarcane Juices
Haze dextran in juices was based on the modified method of Eggleston and Monge (2005).

Mannitol Determined by IC-IPAD
See Eggleston (2002) for method. Dilutions varied, depending on the juice, from 1 g/50 ml to 1 g/500 ml.

Statistics
Single factor ANOVA was conducted using Microsoft Excel[TM], version 2002 with SP-2.

Development of Mannitol as an Indicator of Bacterial Contamination in Fuel Alcohol Production

Growth of Bacterial Isolates

Bacteria, in freeze-dried form, were from the Fermentec (Piracicaba, Brazil) industrial collection. They were reactivated in MRS medium (24 h at 32° C) and then grown for 48 h at 32° C in cane juice medium (containing 2.5% reducing sugars) supplemented with 1% yeast extract. Bacterial counts were performed either by plating or optical microscopy. Mannitol in the growth mixtures was measured by IC-IPAD.

Yeasts

Two *Saccharomyces cerevisiae* strains (BG-1 and PE-2, from the Fermentec collection) commonly used in industrial fermentation were propagated under anaerobic conditions at 32° C using molasses medium with 8% total sugar, doubling the medium volume each 24 h until there was sufficient biomass for the assay, which was collected by centrifugation (20 min, 800 x G).

Mannitol Determined by IC-IPAD

Mannitol was measured on a Dionex DX-300 system.

Alcohol Fermentation

Fermentation was performed at 33° C in 150 ml centrifuge vials containing 8 g of yeast (fresh weight with 25% dry matter) and 80 ml of substrate (a mixture of cane juice and molasses containing 18% total sugar, each source representing 50% of the sugar). Two different strains of *Saccharomyces cerevisiae* (BG-1 and PE-2) were used in separate experiments (in duplicate) with induced bacterial contamination (*Lactobacillus fermentum* inoculated during the first fermentation at different levels: 2×10^6, 1×10^7, and 5×10^7 cells/ml). After fermentation (8-10 h) the fermented medium was centrifuged (800 G, 20 min) and the yeast and bacteria settled were fed again with substrate, performing two additional fermentations cycles. Bacterial counts (microscopy) and mannitol content were measured in the fermented media at the end of the last two fermentation cycles.

Results and Discussion

Development of Enzymatic Method to Measure Mannitol

Stability of the Enzyme

Initially there was a problem with the stability of the enzyme which had been diluted in a phosphate buffer without glycerol, even if the stock or diluted enzyme was stored in a -40° C freezer. This is illustrated in Figure 2. After 8 days of storage in a -20° C freezer the MDH activity had markedly decreased from 1.88 to 0.20 U/ml. Stabilization studies were undertaken, and it was found that the addition of glycerol to the buffer stabilized the enzyme stored as both stock or as a further diluted solution. There was a slightly higher level of stability of the enzyme diluted from stock that had been stored in buffer containing 30% glycerol, compared to that from stock that contained no glycerol, and this increased stability was observed even in fresh, unstored preparations (day 1). As can be seen in Figure 3, both the preparation of the enzyme stock, and diluted enzyme from the stock, need to be undertaken in phosphate buffer with glycerol. Glycerol at the 30 % level was found to be optimum (Figure 3). The freeze dried enzyme is stable in a -20° freezer for up to 6

months. The enzyme stock and diluted enzyme can be stored in conventional -20° C freezers.
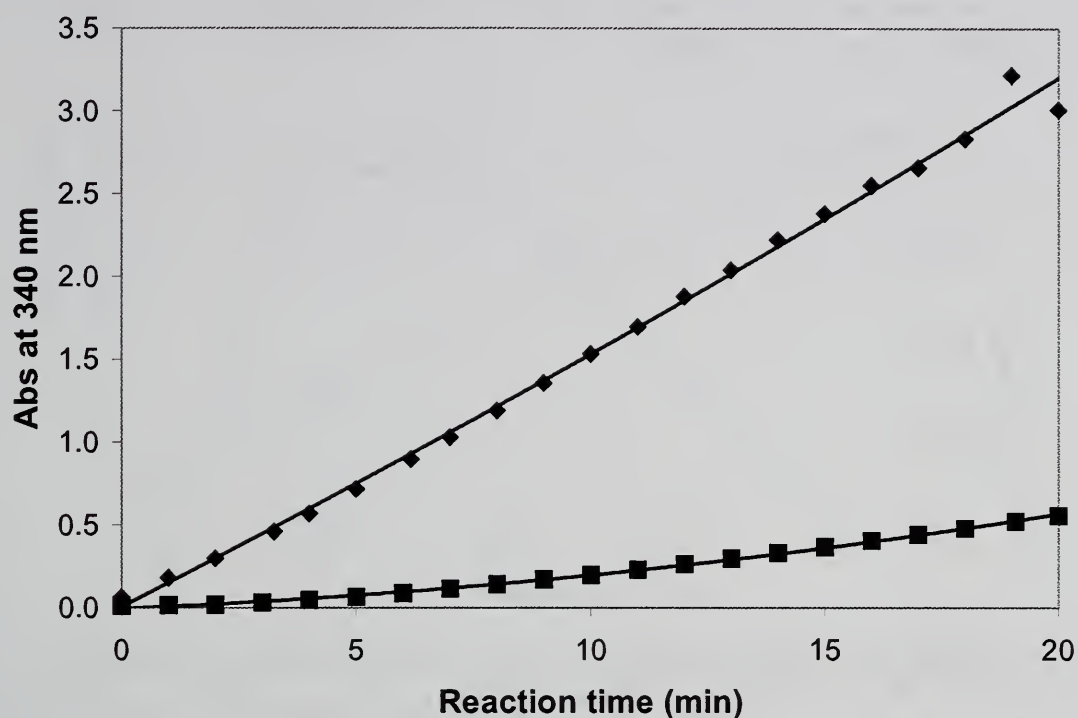
Figure 2. Instablity of MDH when the stock enzyme solution was prepared in phosphate buffer only with no added glycerol.
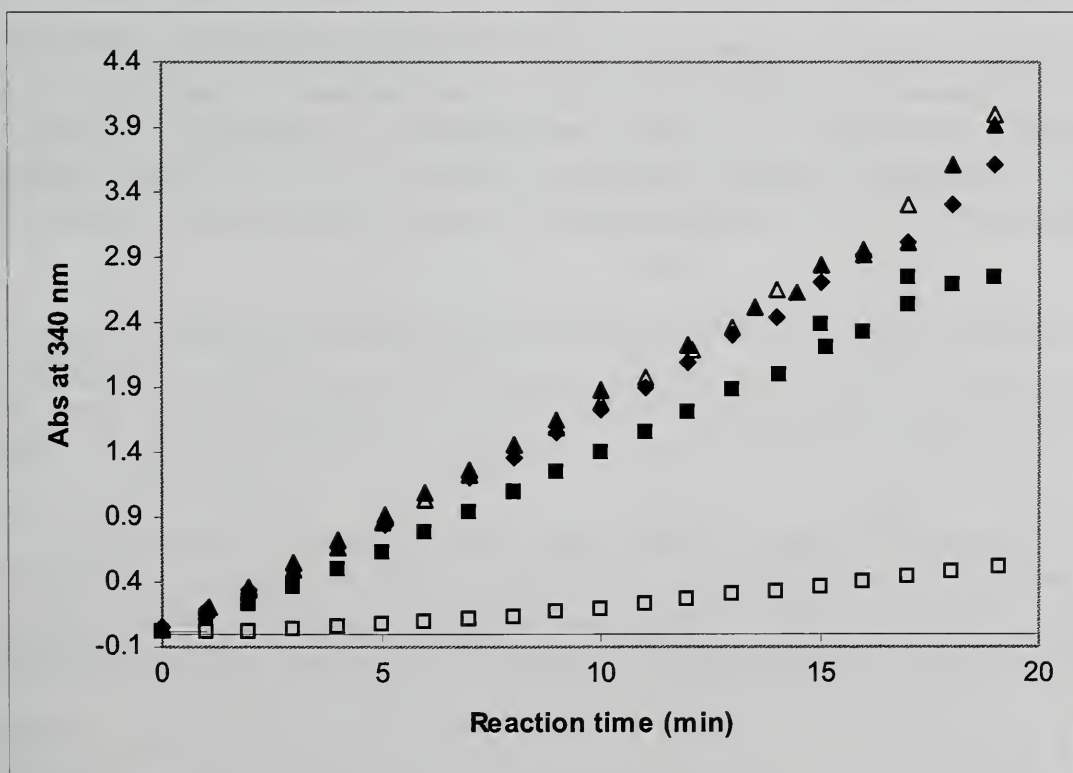
Figure 3. Stabilization of MDH after 8 days when the stock solution was prepared in phosphate buffer, with added glycerol at different concentrations.

Effect of Temperature

In early efforts to develop a simple method to determine mannitol in sugarcane consignments, there was concern that the method was relatively rapid. For this reason, we investigated the effect of temperature on MDH activity – see Figure 4.
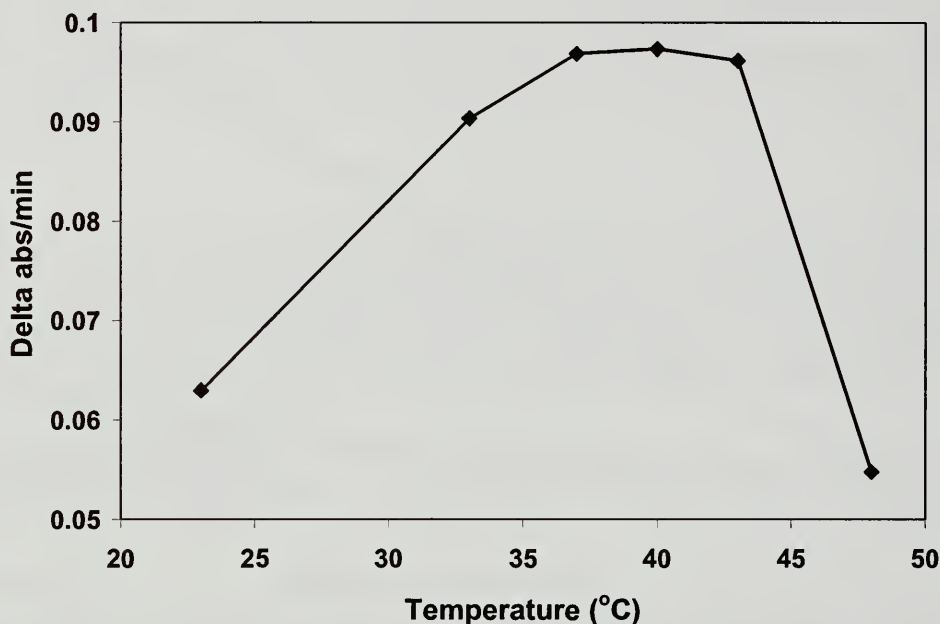
Figure 4. Effect of temperature on the activity of mannitol dehyrogenase (MDH)

Although the activity of MDH is optimum between 37-43° C (Figure 3), incubating the enzyme/juice mixture in a waterbath at the factory would only add another level of complexity and cost. We therefore decided to keep the reaction at room temperature (23° C) for the final factory method. Nevertheless, if factory staff wanted an even faster method, they can incubate the enzyme/juice mixture in a waterbath at 40° C for 2 min, instead of leaving it at room temperature for 5 min.

Linearity

The relationship between the mannitol concentration and the absorbance at 340 nm after 5 min was found to be only approximately linear up to 1000 ppm, which may be due to lack of substrate at much lower levels, and product inhibition ~1000 ppm level. Therefore, up to the 1000 ppm level, a quadratic fit would be better. A better linear fit was found from 1-500 ppm mannitol (Figure 5).

Precision of the Enzymatic Factory Method to Determine Mannitol in Cane Juices

The method precision was very acceptable in four cane juices assayed (Table 1), with coefficients of variation ranging from 1.73 - 5.13%. The precision tended to become worse when relatively lower amounts of mannitol were present in slightly deteriorated and undeteriorated cane juices (Table 1).
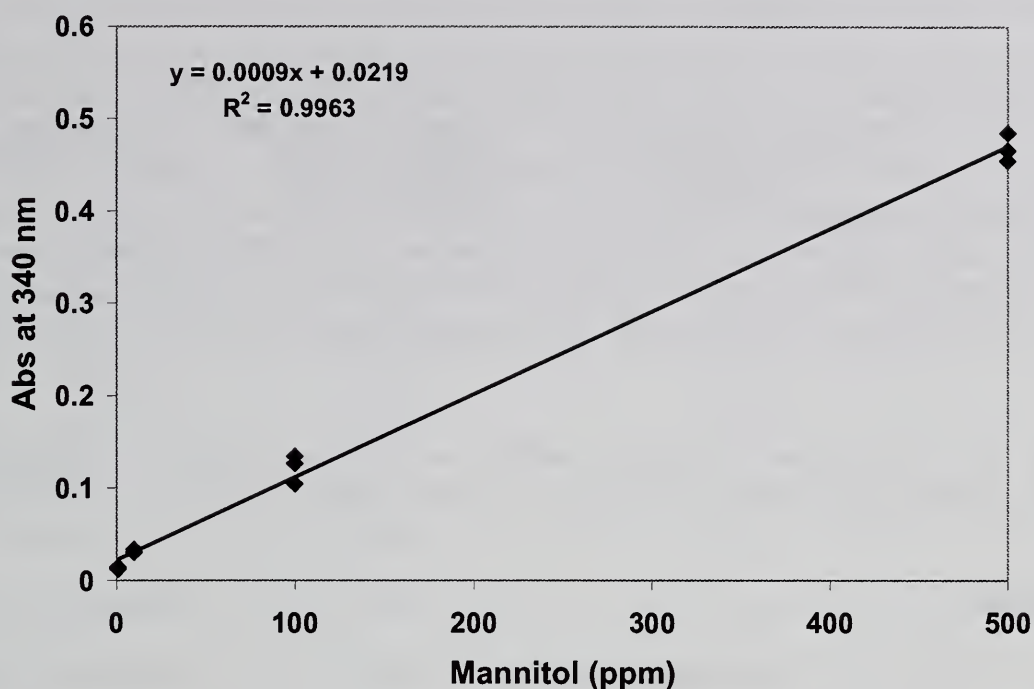
Figure 5. The linear relationship between mannitol concentration and absorbance at 340 nm after 5 min incubation at room temperature.

Table 1. Precision of the enzymatic method for the determination of mannitol in sugarcane pressed juices expressed as the coefficient of variance (CV).

Cane juice sample	Juice °Brix	N	Mean concentration of mannitol (ppm)	Mannitol variation CV (%)	Haze dextran (ppm)
Deteriorated, pressed cane juice[a]	14.89	10	20,455	3.32	4,688
Crusher juice from Factory A	14.70	8	3,870	4.50	585
Crusher juice from Factory B	16.01	10	3,259	5.32	204
Crusher juice from Factory C	14.67	9	7,180	1.73	944

[a] Had to be diluted 8-fold

Determination of Mannitol in the Presence of Other Sugars in Cane Juice

Sugarcane juices have high levels of glucose, fructose, and particularly sucrose. Dextran will also be present when mannitol is present in cane juice because both are formed from Leuconostoc (Eggleston, 2002). Short and long chain carbohydrates could potentially interfere with the measurement of mannitol. We, therefore, investigated the effect of different sugars as they approximately occur in sugarcane juice, using simulated, model juice and results are shown in Table 2. None of the sugars showed any statistical interference at the 95% probability level with the mannitol determination, which confirms the ability of the method to accurately measure mannitol in cane juices. The analytical recoveries were very acceptable between 99.1-104.2% (Table 2).

Accuracy

The accuracy of the developed enzymatic method to measure mannitol in cane juices was determined by comparing the results with an ion chromatography (IC-IPAD) method (Figure 6).

$$y = 1.0068x + 8.7103$$
$$R^2 = 0.9992$$
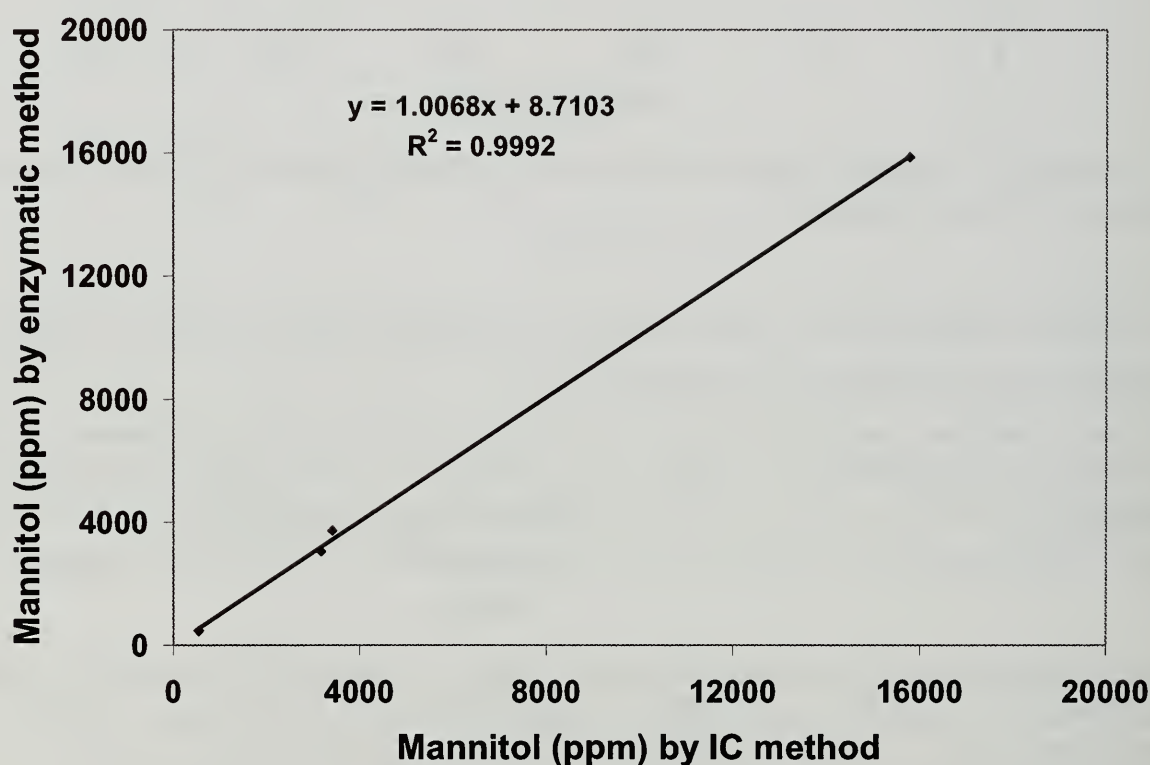
Figure 6. Linear correlation between the enzymatic method for determining mannitol in cane juice and an ion chromatography (IC) method. No statistical differences between the two methods were found at the 95% probability level for any sugarcane juice studied.

An excellent correlation existed ($R^2 = 0.99$) between the two methods, validating the accuracy of the enzymatic method. Furthermore, there were no significant differences at the 95% probability level, between the two methods for any of the juices analyzed. Precision was, however, worse for both methods in the juice containing <550 ppm mannitol (Figure 6), which reflects the difficulty of detecting small amounts of mannitol, and confirms results listed in Table 1. As factory staff are more concerned with detecting mannitol concentrations >550 ppm in deteriorated pressed cane juice at the factory, this lower precision should have limited impact at the factory.

Table 2. Determination of mannitol in the presence of other sugarcane sugars in simulated 14.0 °Brix juices.

Simulated juice[a]	Mannitol conc. added (ppm)	Mean % recovery of mannitol[b, c]	Total variation CV (%)[c]
Sucrose	2000	100.6	1.86
Sucrose + glucose	2000	102.5	3.16
Sucrose + glucose + fructose	2000	99.6	3.28
Sucrose	1000	103.6	2.97
Sucrose + glucose	1000	104.2	3.26
Sucrose + fructose	1000	103.6	1.36
Sucrose + glucose + fructose	1000	99.1	7.16
Sucrose + glucose + fructose + dextran (diluted 1:2; 7.0°Brix)	1000	100.5	1.63

[a] See Materials and Methods section
[b] N=3
[c] No statistical differences were found amongst the simulated juices with different sugars added using single factor ANOVA

Relationship of Mannitol with Dextran Measured by the Haze Method
As well as mannitol concentrations, Haze dextran concentrations in four cane juices are shown in Table 1, and the correlation between Haze dextran and mannitol was excellent at $R^2 = 0.99$ at the 99% probability level. This confirms previous observations from a laboratory cane deterioration study (Eggleston, 2002). In more complex field studies of cane deterioration (Eggleston and Legendre, 2003; Eggleston, *et al.*, 2004), other strong correlations were found between mannitol measured by IC and dextran, but with slightly lower R^2 values of 0.84, which reflects the further complexity of the multiple field samples representing numerous cane varieties, and possibly other bacterial sources of sugarcane deterioration (see Table 3).

As seen in Table 1, mannitol concentrations were markedly higher than Haze dextran concentrations. Higher mannitol than dextran concentrations have been previously observed in deteriorated juices from different cane varieties (Eggleston and Legendre, 2003; Eggleston, *et al.*, 2004) and in juices from Rhizoctonia affected beets that were susceptible to Leuconostoc deterioration in the late stages (Bruhns, *et al.*, 2004). This not only highlights the usefulness and higher sensitivity of mannitol to better predict Leuconostoc and other bacterial cane deterioration (see Table 3) than dextran, but the underestimation by sugar industry personnel of the relatively large amounts of mannitol present in

deteriorated cane that can affect processing.

Development of Mannitol as an Indicator of Bacterial Contamination in Fuel Alcohol Production

Mannitol in Contaminating Bacteria
Numerous types of bacteria can contaminate yeast fuel ethanol fermentations. Lactic acid bacteria, Leuconostoc and Lactobacillus, are common contaminants and frequently associated with process problems. Lactobacillus is adapted to the alcoholic and nutritional conditions of the process (Oliva-Neto and Yokoya, 1994), but Leuconostoc is more sensitive to alcohol and usually does not persist as long. The lactic acid formed by such bacteria can strongly inhibit yeast metabolism and decrease alcohol yield.

Known contamination bacteria were grown on a medium of sugarcane juice to evaluate their ability to produce mannitol, and results are listed in Table 3.

Table 3. Formation of mannitol by bacterial isolates from industrial processes.

Bacteria	Mannitol (ppm)	Plating Counts (CFU x 10^7/ml)
Acetobacter pasteurianus	<50	71.0
Bacillus coagulans	<50	2.8
Bacillus megaterium	<50	1.0
Bacillus subtilis	<50	2.0
Bacillus stearothermophilus	<50	2.0
Lactobacillus buchneri	1070	15.0
Lactobacillus fermentum	5110	4.8
Lactobacillus fructosus	5970	3.4
Lactobacillus vaccinostercus	1875	16.0
Leuconostoc mesenteroides	7480	8.3

Mannitol production depended on the bacterial species and strain. The highest producer of mannitol was *Leuconostoc mesenteroides*, which supports the evidence that mannitol is a sensitive indicator of sugarcane (Eggleston, 2002; Eggleston, *et al.*, 2004) and sugarbeet deterioration (Thielecke, 2002). Furthermore, it was the common contaminant of fuel ethanol fermentations from *Lactobacillus fermentum* and *fructosus* that produced the most mannitol (Table 3). This suggested that mannitol would be a useful indicator of bacterial contamination. More importantly, it also explained why ethanol yields are sometimes lower than expected with respect to the acidification of the fermentation medium, because these bacteria competed with the yeast for substrate to produce mannitol rather than ethanol.

Mannitol as an Indicator of Bacterial Fermentation in Alcohol Fermentation
To verify if mannitol could be an indicator of bacterial fermentation, an experiment was conducted to measure mannitol in fermentations performed with induced bacterial contaminants, and results are illustrated in Figure 7. A good correlation existed between mannitol and the bacterial cell count, proving that mannitol was a valid indicator.
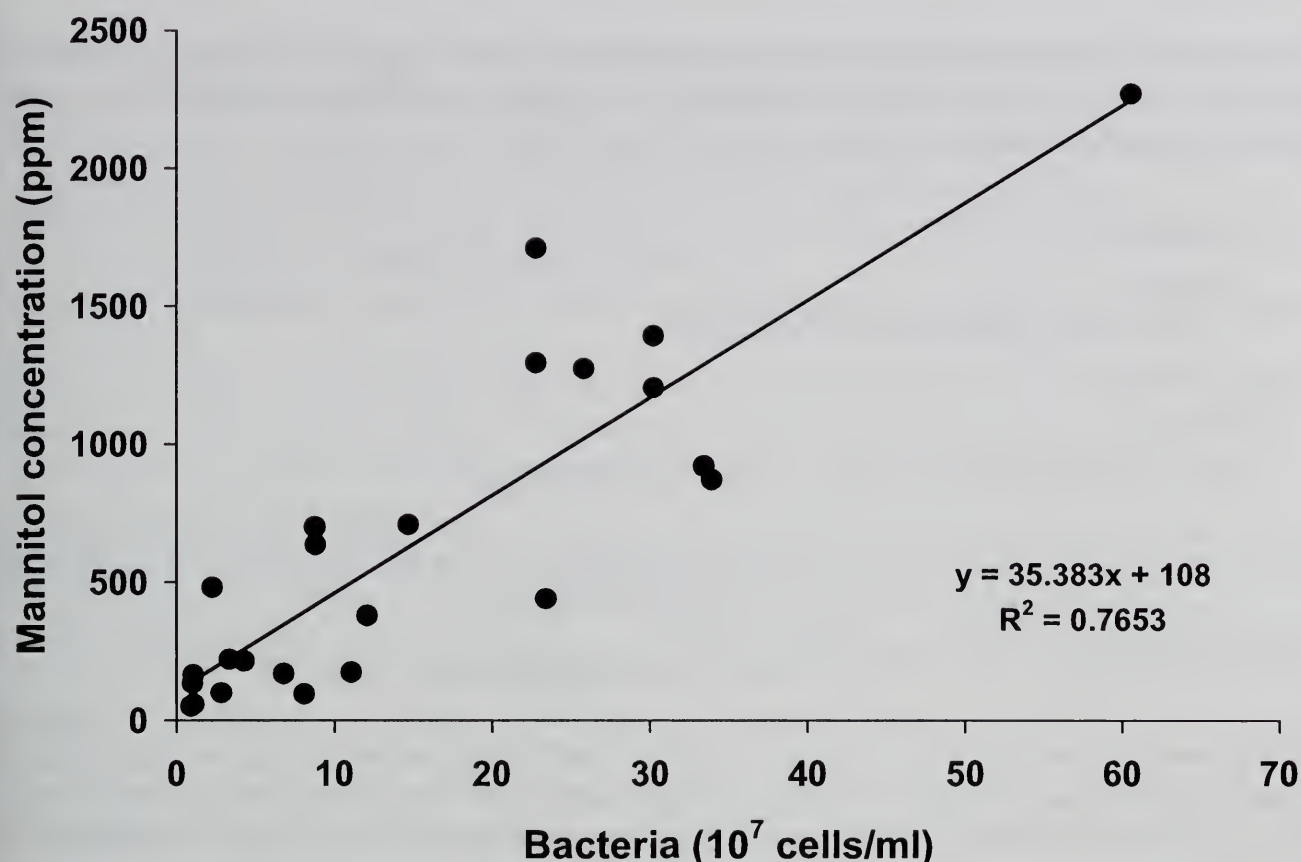
Figure 7. Relationship between bacteria grown in alcoholic fermentation and mannitol formation. Fermentation was performed with induced bacterial contamination.

Advantages of Mannitol as an Indicator of Bacterial Fermentation
Mannitol serves as a very important complimentary method to other direct and indirect methods to monitor bacterial contamination in sugarcane processing to fuel alcohol production:

1) Mannitol is not produced by the starter strain or by wild *Saccharomyces cerevisiae* yeast.

2) Mannitol is produced (mannitol at the end of fermentation – mannitol in the imput must and yeast) by bacteria during fermentation and can account partly or wholly for decreased ethanol yields. Approximately 6000 ppm mannitol can cause an approximate 4% drop in ethanol yield.

3) Mannitol is easily measured.

Conclusions

An enzymatic method has been developed to measure mannitol and, therefore, the extent of deterioration in juice pressed from consignments of sugarcane delivered to the factory. This enzymatic method has many advantages for the factory staff:

✓ Simplicity

✓ Rapid

✓ Uses existing equipment at most factories

✓ Accurate

✓ Precise

✓ Not affected by the presence of other sugarcane sugars

✓ Only ~60 U.S. cents per analysis (largest cost is the NAD at 45 cents; MDH cost is12.5 cents per analysis) that is much lower than the cost for rapid dextran analysis by monoclonal antibody technology (Rauh et al, 2001)

✓ Mannitol is a more sensitive indicator of sugarcane deterioration than dextran.

Further research needs to be undertaken to verify if the method can be used to measure bacterial contamination in fuel alcohol production. A bright purple color forms when the enzymatic method is used to measure mannitol in deteriorated sugarbeet juice; research is needed to ensure that this color does not interfere with the determination of mannitol in sugarbeet juice.

Mannitol has been shown to be an indicator of bacterial contamination in fuel alcohol fermentations that can complement other indicators and methods to monitor contamination:

✓ It is not produced by the starter or wild strains of *Saccharomyces cerevisiae* yeast

✓ It is produced by bacteria during fermentation

✓ It can account for unexpected alcohol yield drops

✓ It is easily measured

Not all contamination bacteria produce mannitol so other monitoring techniques are also needed for a full picture. Future research is planned to evaluate mannitol as a bacterial contamination indicator further using other bacteria isolated from industrial processes.

Acknowledgements

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Delay Between Sugarcane Harvesting and Crushing: Aconitic Acid Ratio as a Physiological Aging Indicator

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Introduction

The worldwide sugarcane industry is subject to delays between cane harvesting and crushing. Sugarcane deterioration is well documented on burnt cane. Robillard (1990) and Cox (1992) found between 0.42 to 1.5% loss of sugarcane weight per day. Post harvest deterioration process is known to be pronounced on burnt cane with relative sucrose weight lost between 1.3% (Cox, 1992) and 2.3% per day post harvest (Robillard, 1990). Many different deterioration indicators have been studied: Ethanol (Cox, 1992), dextran and mannitol (Eggleston, 2005). Green cane deterioration is less documented; a few indicators like pH have been described (Sens, 2005).

In Reunion Island, strict environmental regulations forbid cane burning. For Reunion's sugar industry, a better understanding of sugar loss in green cane is a major issue.

Objectives

- Describe experimental "deterioration" trials on green sugarcane:
    - Loss of sugar,
    - Find an aging indicator for post harvested cane,
- Link experimental deterioration trials to factory mixed juice results.

Figure 1: Degradation of sugarcane in a field.

Materials and Methods

Deterioration Trials
- 16 trials in different agro-environmental conditions,
- 300 kg of green sugarcane left 15 days in field,
- Every day:
  - ➢ The same 10 sugarcanes are weighed,
  - ➢ Analysis of 10 other sugarcanes:
    Pol in cane and fiber content,
    Aconitic Acid (juice was frozen).

Weekly Mixed Juice Sample
- Sampling every 2 hours, frozen; Le Gol Mill, 5 milling tandem.

Analytical Methods
- Pol in cane (R') measure in Press Juice (1'30, 200 bars),
- Reunion's formula: $R' = Sj \times (1-m/c) / E$,
  - ➢ Sj = Lead Pol in Juice,
  - ➢ m = weight of the press fiber cake,
  - ➢ c = weight of cane in the press,
  - ➢ E = press extraction = $1.012 - 0.41 \times (m/c)$,
- Aconitic Acid quantified with :
  - ➢ Waters HPLC, UV detector,
  - ➢ KC811Shodex column, eluent phosphoric acid.

Calculation
- Relative sugarcane weight,
  = Weight Dayx / Weight Day0
- Relative sucrose weight,
  - ➢ Expressed as percentage of mass in fresh cane,
  = Weight Dayx × R'Dayx / Weight Day0 × R' Day0

Results and Discussion

Deterioration Trials
- Per day after harvesting: Pol in cane lost 0.11%, purity drops of 0.93 point
  - ➢ High standard deviation, due to sugarcane heterogeneity

- A mean loss of 0.98% of sugarcane weight per day due to: (Figure 2, left)
  - ➢ Water evaporation
  - ➢ Sucrose loss

- Loss of 1.6% of relative sucrose (Pol) weight per day after harvesting. (Figure 2, right)

Aconitic Acid Ratio: A Post Harvest Age Indicator of Sugarcane
- Aconitic acid has 2 isomers:
  - Trans-Aconitic,
  - Cis-Aconitic.

- After harvesting, the Cis / Trans Aconitic ratio increases: (Figure 3, left)
  - Trans-Aconitic isomerizes to Cis-Aconitic
  - Less with low temperature (Altitude >700 m),
  - Strongly with high temperature (Coastal Zone).

- The ratio is well correlated to true purity. (Figure 3, right)

Weekly Mixed Juice sample
- Aconitic Acid Ratio was not correlated to weekly Mixed Juice Purity (Figure 4, left)
  - Because MJ Purity increased during the crushing season.

- Acid Trans Aconitic was correlated with a chemical benchmark: ET1 (Figure 4, right)
  - Farmers deliver green cane to delivering station,
    - ✓ Millers buy sugarcane in delivering station,
  - Millers crush sugarcane,
    - ✓ ET1 = Lost between buying and crushing, % cane.

- Aconitic Acid Ratio increased in special conditions :
  - 1: 2003, Low mixed juice purity
  - 2: 2003, strike in cogeneration plants starts week 43
  - 3: 2004, strike in cogeneration plants starts week 43

Conclusions

- After green harvesting, sugarcane lost:
  => 0.98% of weight per day,
  => 1.6% of sucrose weight per day.
- Green cane deterioration is lower than burnt cane deterioration.
- Aconitic Acid seems to be linked to a benchmarking indicator (ET1).

Further Work to Be Conducted
- Deterioration trials in a wide range of agro-environmental conditions.
- More data needed to explain aconitic acid isomerisation.
- Explain metabolism pathway of sugarcane deterioration (Krebs Cycle).
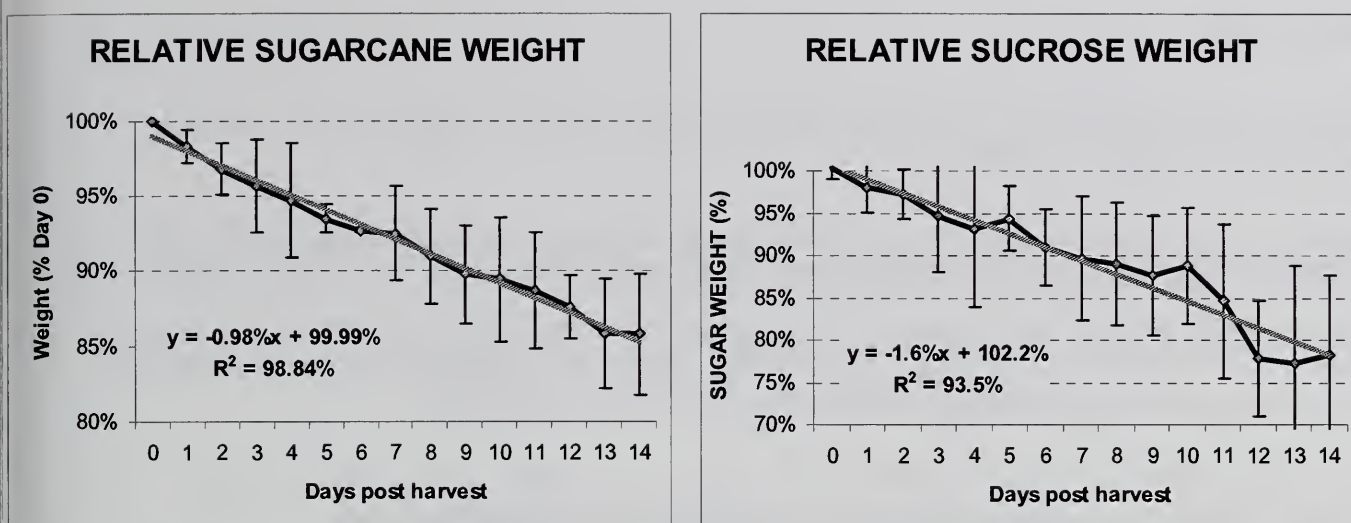
Figure 2. Relative sugarcane weight (left) and relative sucrose weight (right).
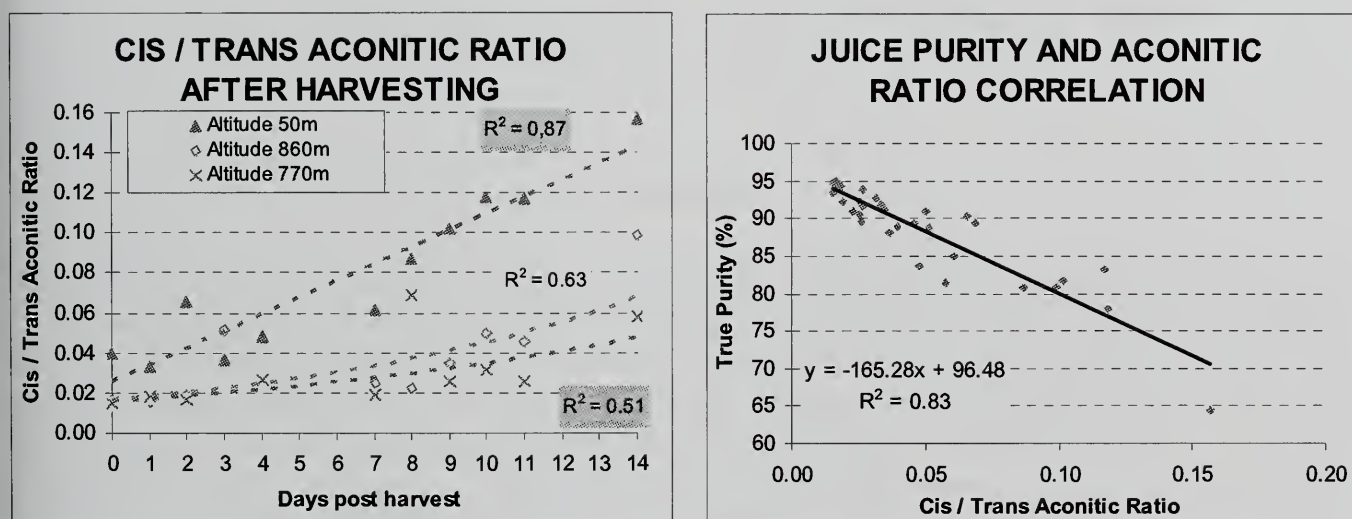
Figure 3. Cis/Trans aconitic ratio after harvesting (left) and juice purity and aconitic ratio correlation (right).
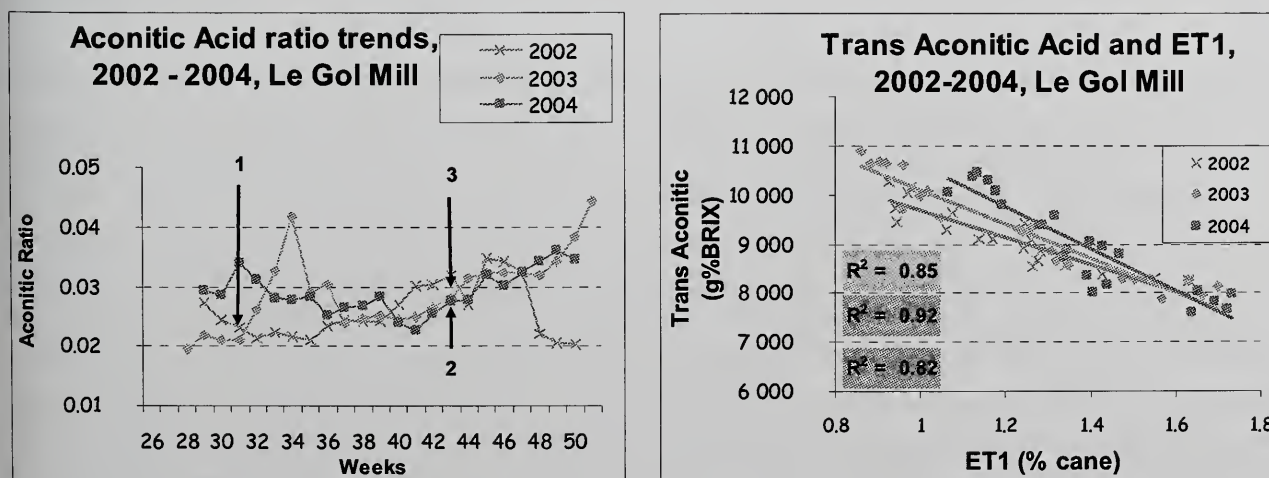
Figure 4: Aconitic acid ratio trends (left) and Trans aconitic acid and ET1 (right).

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Second Year of Results for the Application of Rendement Factors Concept to Predict Sucrose Losses to Molasses from Mediterranean Sugar Beet

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Abstract

In 2004, for the first time a new quality formula was established for Moroccan beet using the Rendement Concept of Burba and Harling (2003). During the 2005 campaign, additional analyses of molasses, thick juice and cossettes, taken over a period of 40 days from the same Moroccan sugar factory, were carried out to verify the data and the formula. The formula for 2005 is structurally similar to the formula from 2004. The only variation observed concerns the ratio Total Soluble Nitrogen / Alpha Amino Nitrogen. The Moroccan Rendement Formula, established over two campaigns, allows the assessment of the relative technical beet quality based on standard tare house analyses (K, Na, amino-N). This concept avoids the effort to produce thick juice or molasses in a pilot plant.

Introduction

Culture of sugar beet was introduced in Morocco in the sixties and is distributed today in five regions; the quantity of sugar produced by the different sugar factories, from beet (and from cane in the north of the country) cannot satisfy the national need which is 30 kg/person; about 500,000 tons of sugar are imported every year. Furthermore, in spite of a good sucrose content (15 - 18%) and a satisfactory root yield (45-60 t/ha), the technological beet quality remains not optimal with respect to invert sugar, sodium and nitrogen (Fares, *et al.*, 1996; Fares, *et al.*, 2004*)*. This lower quality can be explained by excessive nitrogen fertilization (Moughli, *et al.*, 1990; Fares, *et al.*, 1995a), the quality of irrigation water in some regions (Ghoulam and Fares, 2001), deficiency in certain elements, such as boron, and by beet storage under high temperatures in summer (Fares, *et al.*, 1995b). Unsatisfactory beet quality is a common problem in the south of Spain, southern Italy, Greece and other countries in North Africa (Gordii Ingelmo and Morillo Vellarde, 1990; Sanz Saez, 1990; Maslaris and Christodoulou, 1992; Fares, *et al.*, 1996; Salvo Salanova, 1999).

Technical beet quality is assessed by different quality formulas which are based on different quality concepts, which allow the estimation of sucrose losses to molasses from beet analysis (Figure 1), either via thick juice analyses (e.g. Ruiz-Holst, *et al.*, 2002), experimental molasses (e.g. Buchholz, *et al.*, 1995), or fixed sugar - nonsugar ratios in molasses ("Rendement Factors", e.g. Andrlik, 1904 / 05; Dedek and Ivanchenko, 1926; Wieninger and Kubadinow, 1972; Devillers, 1988; Huijbregts, 1999; and others). A selection of formulas obtained are summarised in Table 1.
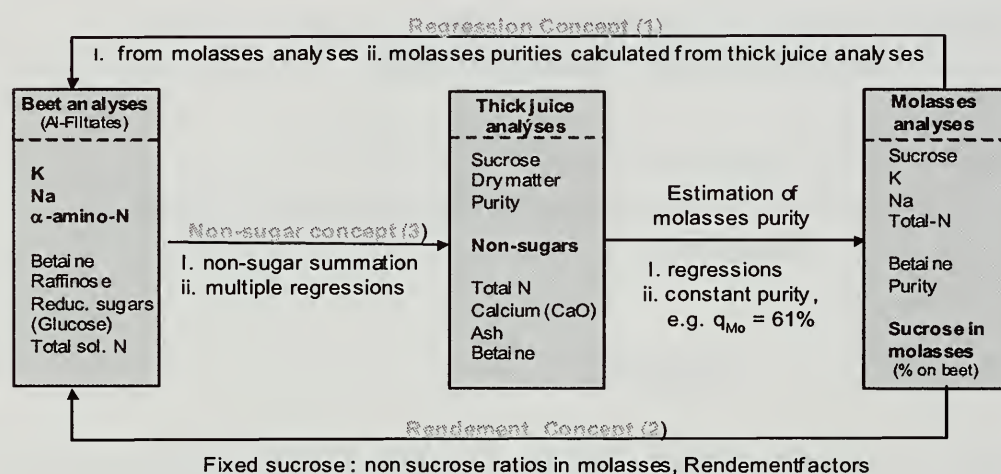
Fig. 1: Concepts for prediction of sucrose losses to molasses.

In Morocco some sugar factories are making further analyses on beet at the receiving station so they can have the tools to advise the growers and to improve beet quality. Different formulas for the prediction of sucrose losses to molasses (Sm) are available in Morocco:

Sm = 0.14 (Na + K) + 0.25 αN + 3.3 Glucose + 0.3   (Devillers, 1988)
with Sm in g/100 g beet, Na, K, αN in mmol/100g beet, Glucose in g/100 g beet.

Sm = 0.061 Na + 0.018 K + 0.019 αN + 13.998   (R'Zina, 1994)
with Sm in g/100g sucrose, beet composition in mmol/100 g sucrose.

Sm = 0.16 K + 0.34 Na + 0.091 αN + 5.67   (Hachimi, *et al.*, 1990)
 and
Sm = 0.13 K + 0.34 Na + 0.0041 αN+ 0.0044 Invert + 5.61
with Sm in g/100 g sucrose, Na, K, αN in mmol/100 g sucrose and invert in mg/100 g sucrose.

Sm = 0.18 (Na + K) + 0.2 αN   (Iserf, 1989)
with Sm in g/100g beet, beet composition in mmol/100g beet.

Table 1: Selected formulas for beet quality assessment.

Quality Formulas	References
i. based on molasses analyses	
$m_{S,Me} = 0.14 \cdot w'_{(K+Na)} + 0.25 \cdot w'_{\alpha N} + 0.60$	Devillers, 1988*
$m_{S,Me} = 0.12 \cdot w'_{(K+Na)} + 0.24 \cdot w'_{\alpha N} + 0.48$	Buchholz et al., 1995
$m_{S,Me} = 0.11 \cdot w'_{(K+Na)} + 0.23 \cdot w'_{\alpha N} + 1.10$	Bubnik and Kadlec 1997 / 2002
ii. based on thick juice analyses (molasses purity predicted from these data)	
$m_{S,Me} = 0.15 \cdot w'_{(K+Na)} + 0.28 \cdot w'_{\alpha N} + 0.37$	Akyar et al., 1980
$m_{S,Me} = 0.15 \cdot w'_{(K+Na)} + 0.22 \cdot w'_{\alpha N} + 0.20$	Tschernjawskaja and Chelemski, 1993

*: Glucose was included in the constant using an average value of 0.5 mmol/100 g beet

However, the Moroccan equations were not obtained under optimal conditions. Deviller's equation, which is mainly used in Morocco today, was established in Europe and possibly does not correspond to the local beet quality. Furthermore, these formulas give different results with variation as a function of the region (R'Zina, 1994). The application of the Rendement Concept, which relies on fixed sugar-nonsugar ratios in molasses (Rendement Factors, Burba and Harling, 2003), could have some advantages for establishing Moroccan quality formulas. This concept does not need costly laboratory work on thick juice or experimental molasses but only standard analyses on factory molasses. The concept allowed Burba and Harling (2003) to propose an equation for the prediction of sucrose in molasses from beet analysis:

Sm = 0.15 (K + Na) + 0.28 αN + 0.25   [% on beet]
(Beet compounds in mmol/100 g beet)

This work applies the Rendement Concept to Moroccan beet with the aim to establish a beet quality formula specifically adapted to the Moroccan conditions.

Materials and Methods

1. Sampling of beet, juices and molasses

For two campaigns (2004 and 2005), samples of beet and the corresponding thick juices were taken over 40 days in one Moroccan sugar factory. Each day, six samples of sugar beet slices and thick juice were taken every 2 h, immediately stored, and then mixed to obtain the average day

sample. Molasses was sampled during the 40 days, two times a day. For each day, the two samples were mixed to have the average day sample.

2. Chemical analyses on beet, juices and molasses

Beet and thick juice were analyzed for sucrose by polarimetry, alpha amino nitrogen by the blue number method, total soluble nitrogen (taken as Nharm = noxious nitrogen) in the beet digest and total nitrogen in thick juice by the Kjeldahl method, sodium and potassium by flame photometry, and reducing sugars by the Berlin Institute Method. Technical molasses was analyzed for sucrose, total nitrogen, sodium and potassium using the same methods.

3. Application of the Rendement Concept

A Rendement formula was established according to Burba and Harling (2003) including the following steps:

a- Calculation of molar ratios of sucrose to non-sugars in molasses
(Rendement Factors, R'_N and R'_{K+Na}),

b- Calculation of sucrose losses to molasses (Sm) using this Rendement Factors,

c- Calculation of Sm based on beet analyses using transition coefficients,

d- Substitution of Nharm in beet by amino-N in beet by means of an average factor.

To extend and complete quality assessment of sugar beet by using Rendement formulas, the fraction of molasses sucrose formation (in % on beet) due to the melassigenic properties of invert sugar degradation products and to the addition of neutralizing agents in case of negative ion balance need to be considered separately.

Results and Discussion

1. Application of Rendement Factors to calculate sucrose losses to molasses

From the composition of technical molasses (Table 2) we calculated the Rendement Factors (R'_N and R'_{K+Na}). The mean values are $R'_N = 1.20$ and $R'_{K+Na} = 1.015$. This is in good agreement with data published in the literature (Burba and Harling, 2003).

Table 2. Average composition of technical molasses from a Moroccan sugar factory (2004 and 2005campaigns).

	Sucrose (%)	Brix (%)	Purity (%)	Total Nitrogen (mmol/100g)	Potassium (mmol/100g)	Sodium (mmol/100g)
2004	48.13	84.31	57.14	123.74	55.93	84.60
2005	50.35	86.31	58.35	117.15	62.26	81.17

Rendement Factors:

$$R'_N = \frac{\text{Sucrose}}{\text{Total nitrogen}}$$

$$R'_{K+Na} = \frac{\text{Sucrose}}{(K + Na)}$$

Sucrose, Total Nitrogen, K and Na are expressed in mmol / 100 g molasses.

Considering the factors calculated we deduce:

$Sm = 0.342 \times 1.015 (Na + K)$ (% on molasses)
$Sm = 0.342 \times 1.20 (Nharm)$ (% on molasses)

Na, K and Nharm expressed in mmol/100 g molasses.

And after combination of both equations:

$Sm = 0.171 \times 1.015 (Na+K) + 0.171 \times 1.20 (Nharm)$ (% on molasses)   **(1)**

Na, K and Nharm expressed in mmol/100 g molasses

2. Calculation of sucrose losses to molasses from beet analyses

To calculate sucrose losses to molasses based on beet analyses, transition coefficients (beet to thick juice) for Nharm and (K+Na) need to be considered. From the analysis of sucrose, Na, K, and Nharm of beet and corresponding thick juices, the transition coefficients were calculated; the mean values are given in Table 3. For Na and K, the coefficient 0.90 is in the same range as the coefficient (0.81) found by Schiweck, *et al.* (1994). The average transition coefficient for total soluble nitrogen (Nharm) was 0.87. This is also close to published values (Pavlas, 1957).

Thus based on equation **(1)**, Sm can be calculated on beet analyses by employing these transition coefficients:

Sm = 0.171. 1.015. 0.90 (Na+K) + 0.171. 1.20. 0.87 (Nharm)   (% on beet)   **(2)**
Na, K and Nharm are expressed in mmol/100 g beet.

3. Substitution of Nharm by amino-N in beet

Today Nharm cannot be analyzed routinely in the tare house. Therefore, Nharm in beet needs to be substituted by amino-N. In order to do this, a constant factor was calculated. The factor was determined from the mean values of Nharm in beet and amino-N in beet as following:

$$\text{Factor} = \frac{\text{Nharm in beet}}{\text{Amino-N in beet}}$$

Nharm and Amino-N are expressed in mmol/100 g sucrose.

Table 3: Average content of Na, K, amino-N and total soluble nitrogen in beet and thick juices (in mmol/100 g sucrose) and the derived transition coefficients for the two campaigns (2004 and 2005).

		Beet	Thick juice	Transition coefficient
Na	**2004**	34.73	30.84	0.89
	2005	33.35	33.08[a]	0.91[b]
K	**2004**	23.45	20.86	0.89
	2005	23.03	20.79	0.91
Total soluble Nitrogen	**2004**	41.77	36.01	0.86
	2005	43.62	38.49	0.88
Amino-Nitrogen	**2004**	22.79	12.83	0.56
	2005	20.06	14.63	0.73

[a]: Included NaOH addition

[b]: corrected coefficient considering the NaOH addition

The mean values for this factor are 1.83 and 2.17, for the 2004 and 2005 campaigns, respectively. The average is 2.00. Thus, equation (2) can be converted to:

$$Sm = 0.171. 1.015. 0.90 \,(Na+K) + 0.171. 1.2. 0.87 . 2.0 \,(amino\text{-}N) \quad (\% \text{ on beet})$$

Sm = 0.16 (Na+K) + 0.36 (amino-N)   (% on beet)   **(3)**

Na, K and amino-N expressed in mmol/100 g beet

This is the final Rendement formula for Morocco which allows estimation of the average total sucrose losses to molasses from standard beet analyses. This final equation is the preliminary Rendement Formula for Morocco, based on a limited data set of the 2004 and 2005 campaigns.

4. Calculation of the fraction of Sm coming from invert sugar degradation products

A separate consideration of invert sugar is very important since in Morocco, invert sugar concentration in beet is much higher than in North and Central Europe (Figure 2, Burba and Harling, 2003, Table 4). Thus in Morocco, a significant fraction of the total sucrose losses is due to invert sugar. This fraction can be calculated by Sm = 0.43 x Inv [% on beet] (Burba and Harling, 2003) considering the melassigenic effect of the acids produced from invert sugar. Based on the data from the 2004 campaign, this fraction represents 20.6 % of the total sucrose losses to molasses.
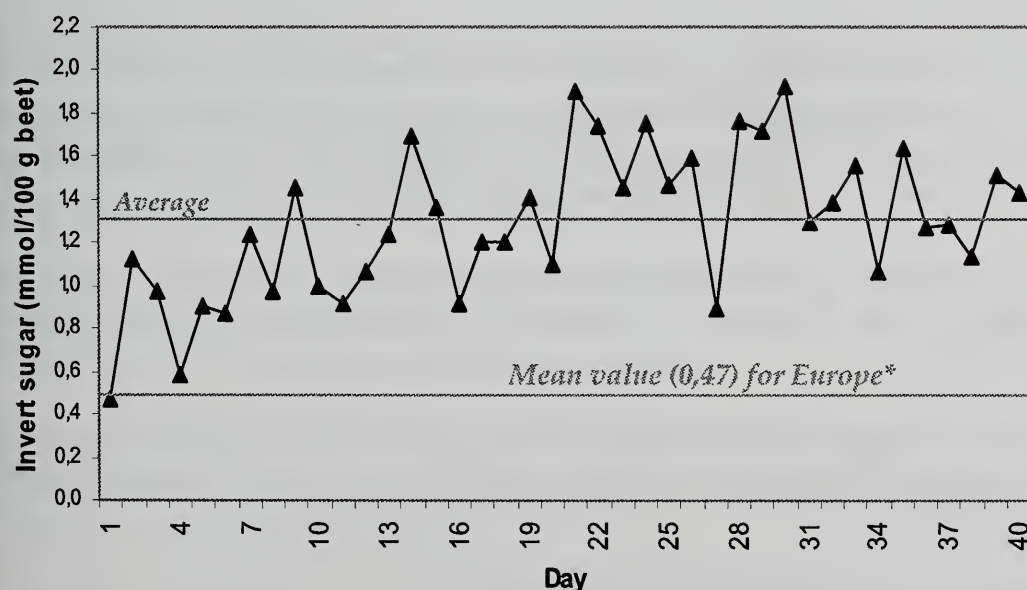
*: according to Burba & Harling (2003)

Figure 2. Invert sugar concentration during 40 days for a Moroccan sugar factory.

The fraction of molasses sucrose coming from soda addition required for neutralisation of juices with insufficient alkalinity reserves, which is also typical for beets harvested in the Mediterranean area, can be estimated: $Sm = 0.305$ /IB/ [% on beet] (Burba and Harling, 2003). However it needs to be proven whether this holds true also for Morocco (Baouch and Fares, 2005).

Conclusion

A new, preliminary beet quality formula for Morocco (Equation 3) was obtained by applying the Rendement Concept of Burba and Harling (2003) to Moroccan sugar beet. This formula is based on technical data from one Moroccan sugar factory during the 2004 and 2005 campaigns. To establish this formula, only standard chemical analyses of cossettes, thick juices, and molasses were required, and no sophisticated technical equipment (e.g. pilot plant) was necessary.

As expected, Equation 3 is structurally similar to other beet quality formulas (Table 1), which are based on different concepts (Figure 1). Equation 3, however, contains no constant. This is due to the facts that (i) the Rendement Factors for (K + Na) and N inherently consider the effects of all other non-sugar compounds present on molasses sugar formation; and (ii) a constant factor had to be employed for the calculation of amino-N in beet from Nharm in beet. As most other quality formulas, Equation 3 allows the estimation of relative sugar beet quality in Morocco, simply based on standard tare house analyses for K, Na and amino-N. It is, however, unique, because it relies solely on material from a Moroccan sugar factory.

The influence of invert sugar on molasses sucrose formation is included in the formula but can be calculated separately.

According to Burba and Harling (2003), calculation of molasses sugar formation due to neutralising agents in the case of an insufficient alkalinity reserve in beets, should be possible. The latter, however, relies on the alkalinity concept of Schiweck and Burba (1993), which remains to be validated for Moroccan beets (Baouch and Fares, 2005).

As all others beet quality formulas, this Moroccan Rendement formula is not eligible to calculate sucrose losses to molasses in the factory. It is intended to use for a relative beet quality assessment before processing.

The results presented are preliminary because they are based on data from one factory and two campaigns. In order to validate the equation, analogous analyses were carried during the 2006 campaign in another sugar factory.

Acknowledgements

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Utilization of NIR Spectroscopy for Factory Control

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Abstract

NIR spectroscopy (NIRS) is an analytical technique that has been expanding into many industries for both analytical and control purposes. NIR is used widely in the food processing and grain industries throughout the world. The sugar industries in Australia, South America, Brazil and Florida are currently using NIR for factory control and/or cane payment. NIR can provide analytical results faster than conventional analyses and can be used as a laboratory instrument or online. The main benefits of NIR are lower labor and chemical costs and more consistent analytical results.

Audubon Sugar Institute and Enterprise Factory in Louisiana worked together to perform a proper evaluation in a factory laboratory to demonstrate the feasibility of a Foss NIR 5000 beverage analyzer for factory control in Louisiana. NIR proved to be at least as accurate as the factory laboratory and capable of providing multiple constituent analyses with one scan.

Audubon personnel prepared the calibrations and evaluated the feasibility of combining sample spectra in order to reduce the number of calibrations and to increase the data range to make the calibrations more reliable for varying samples. Comparisons of the laboratory data and the NIRS data will be presented as well as the calibration comparisons. All data used in the calibrations were obtained from the Enterprise Factory laboratory. A final molasses calibration that included HPLC sugars, conductivity ash, and vacuum oven dry solids was the only exception. The transfer of calibration data from one NIRS to another is one of the challenges of this technology. The final molasses calibration was utilized, but required several bias and slope adjustments using factory data.

Introduction

Like any analytical technique, NIRS has it advantages and disadvantages. Schaffler (2000) lays out some of the advantages of using NIRS for analyzing process streams in a raw sugar factory. Two advantages Schaffler (2000) stated are rapid analyses and the ability to determine multiple analytes with one analysis. Other advantages include no sample preparation other than dilution, no chemical use and less labor required than wet laboratory methods. These advantages can be a significant cost saving for a raw sugar factory.

Disadvantages will offset some of these savings. The upfront cost of an NIRS includes the instrument and the calibration which are a significant investment in both money and time. A newcomer to the NIRS technique will face a steep learning curve as the powerful chemometric software used for calibration requires some practice to acquire the desired results (Schaffler 2000). Several factors contribute to the complexity of the calibration process. NIRS is a secondary method that requires scanned samples must be analyzed by primary methods to build a calibration (Rein 2001). A calibration can be required for each process stream and each calibration requires enough data to be sufficiently robust (Schaffler 2000). The introduction of a new cane variety or major changes in weather introduces new variables that can shift sample spectra out of the calibration model resulting in bias. Thus calibrations will need to be updated routinely. Experienced personnel are required to keep calibrations updated and NIRS errors minimized (Johnson 2000). The transferability of calibration from one instrument to another is not easy. One must run a number of samples on the new instrument to adjust bias and slope for the calibration.

NIR has been used successfully for cane analyses in Australia (Brotherton and Berding 1998) and South Africa (Meyer 1998). In 2001 a trial in Louisiana of the Foss InfaCana NIRS for the direct analysis of cane concluded that the instrument performed adequately (Madsen et al. 2003). At least three InfraCana instruments are in use in the Philippines and Mauritius.

Copersucar field-tested the NIR technique in Sao Paulo, Brazil in 1994 and approved it as acceptable for the analysis of pol and Brix in cane juice (Peterson 1999). Some Florida mills have been using NIR to analyze juice for cane payment (Johnson 2000). American Crystal Sugar Company uses both on-line and laboratory instruments for the analyses of multiple parameters throughout the plant (Jacobson 2000).

NIR is a well accepted quality control method within the international sugar industry. NIR has demonstrated its value in many areas of the sugar industry and will likely continue to expand throughout the sugar industry.

Background

Audubon Sugar Institute (ASI) acquired a demonstration NIRS during the 2000 grinding season. Limited testing was done at St. James sugar mill during the 2000 season, but results looked promising. After the season the instrument was returned to Audubon where final molasses samples were analyzed and a calibration conducted. Again the results looked promising, but more data was needed to build a more robust calibration. The Louisiana Board of Regents

provided a grant for the purchase of a Foss NIR for ASI in 2002. Final molasses data continued to be collected and was used to calibrate the newly purchased instrument. The 2002 season was unique even for Louisiana with the landfall of two hurricanes during the season, followed with weeks of rain added unusual sample data to the calibration.

Through a grant from the American Sugar Cane League and an agreement between Foss North America and the Enterprise Factory, a mill trial in a factory control laboratory was conducted in 2004. The results satisfied the terms of the agreement and Enterprise purchased the Foss instrument. ASI with the support of Enterprise continued to collect data in 2005. Data and calibration information from these two seasons are reported in this paper.

The enterprise factory provided a unique opportunity, since it has both a milling tandem and a diffuser. Diffuser and mill juices are processed separately until the evaporation process where they are combined. Therefore, the control laboratory at the Enterprise Factory analyzes more sample streams than the typical Louisiana factory.

Project Description 2004 Season

1. Summary

This work is a cooperative project between Foss North America, Enterprise Factory and ASI to create calibration packages for the prediction of multiple constituents in mixed cane juice and final molasses by NIR spectroscopic methods.

2. Instrumentation

2.1 NIR

- NIR Systems model 5000
- Beverage module (pathlength1.00 mm +/- 0.02 mm)

2.2 Reference Methods

- Refractometer (Brix)
- Polarimeter (for pol with a 200 mm pathlength and 589 nm wavelength)

2.3 Calibration Development Issues

- Repeatability (REP) Files – to compensate for temperature and instrument variation, a REP file may be created. Note, a REP file was created in 2003 however since both instruments had undergone replacement of key components, this was repeated at the ASI laboratory by Foss North America.

- The instruments were standardized. ASI's NIR is considered the master unit and Enterprise, the host instrument. This is according to the protocol

"Beverage Module Standardization" from 3 June 2004 found in Appendix A. Separate standardization files were created for the molasses and the juice calibration.

3. Samples

3.1 Raw juice and final molasses were used to evaluate the instrument. Other process samples were scanned and control lab data used to develop calibrations for the mill.

3.2 Once calibrations were generated, blind duplicate samples of both juice and final molasses were evaluated to determine laboratory error. These sets consisted of 12 samples not included in the original calibration sets.

3.3 Calibrations were developed for other sample matrices. This was accomplished using the schedule in Table 1. Data was collected weekly by Audubon personnel. During these visits the calibrations were updated with data from the previous week.

Table 1. Sample Schedule for 2004 season

Sample	Sample Freq. per 12 hr shift	Sample count	Samples week
* Raw Juice	4 hr	3	21
Clear Juice	4 hr	3	21
Filtrate	4 hr	3	21
Syrup	3 hr	4	28
A Mol	3 hr	3	21
B Mol	3 hr	3	21
* C Mol	3 hr	4	28
A Mass	3 hr	4	28
B Mass	3 hr	4	28
C Mass	3 hr	4	28
B Magma	4 hr	3	21
Notes:			
* calibrations used in NIR performance evaluation			

4. Constituents

Pol and Brix were used to evaluate the instruments. The performance of the instrument was measured by evaluating pol and Brix on the blind sample tests of raw juice and final molasses.

5. Lab Error Study

To determine laboratory errors, Enterprise and ASI performed lab error studies for mixed juice and final molasses. The results were used to interpret Lab vs. NIR accuracy. The procedure is described in Appendix B. Lab vs. NIR accuracy is considered suitable when the Standard Error of Prediction is less than or equal to 1.5 times the lab Standard Error of Difference. Data from only one of the test is presented in this paper.

Project Description 2005 Season

Calibrations were updated on a weekly basis using data provided by control lab. Some additional samples streams were included and one was dropped due to a change in mill requirements. Additional personnel were used to do analysis on the NIR. Two separate dilutions were used for all diluted samples, each was scanned and one was analyzed by the reference method and the data entered into both data sets. Although this was not the ideal plan for the NIR calibration, it allowed the lab manager to evaluate the NIR data for difference in dilutions. This encouraged greater precision in the dilutions and in the final results. The updated calibrations will be used for the 2006 season.

Results and Discussions

Two blind duplicate studies were done at each lab as described in the experimental plan. Lab and NIR data was suitable in all tests. Data from one test is included in the following tables. Table 2 shows reference data from the Enterprise control lab and the NIR predicted data for the blind sample test of final molasses for Brix. This table also contains the Standard Error of Prediction and the lab Standard Error of Difference for comparison. Identical information on pol data from the same test are displayed in Table 3.

Table 2. Brix data from duplicate blind test for final molasses

Enterprise Lab			NIR		
Dup 1	Dup 2	Abs Diff	Dup 1	Dup 2	Abs Diff
39.80	40.00	0.20	39.77	40.12	0.35
38.90	39.00	0.10	39.02	38.83	0.19
42.30	42.20	0.10	42.25	42.04	0.21
38.00	38.80	0.80	37.58	38.41	0.83
42.60	42.20	0.40	42.37	42.24	0.13
41.60	40.80	0.80	41.72	40.80	0.92
39.70	39.80	0.10	39.94	39.61	0.33
42.30	42.60	0.30	42.15	41.33	0.82
41.40	41.00	0.40	41.17	41.00	0.17
40.10	40.40	0.30	39.79	39.22	0.57
40.30	40.90	0.60	40.63	41.03	0.40
41.60	41.10	0.50	41.39	41.32	0.07
Average	40.73	**0.38**	Average	40.57	**0.42**
			Bias	**0.15**	
Lab Standard Error of Difference				0.38	
Standard Error of Prediction				0.41	

Table 3 Pol data from duplicate blind test for final molasses

Enterprise Lab			NIR		
Dup 1	Dup 2	Abs Diff	Dup 1	Dup 2	Abs Diff
13.20	12.80	0.40	12.45	11.51	0.94
11.20	11.20	0.00	12.26	12.32	0.06
11.60	10.80	0.80	11.85	11.18	0.67
11.60	13.00	1.40	13.38	12.72	0.66
12.00	12.00	0.00	12.03	12.25	0.22
12.80	12.40	0.40	13.68	13.60	0.08
10.40	10.00	0.40	10.06	10.26	0.20
12.80	14.40	1.60	12.59	13.26	0.67
11.20	11.20	0.00	11.47	12.08	0.61
11.80	11.80	0.00	11.05	11.14	0.09
12.00	12.40	0.40	12.07	12.85	0.78
14.00	12.80	1.20	13.89	13.39	0.50
Average	12.06	**0.55**	Average	12.22	**0.46**
			Bias	**-0.16**	
Lab Standard Error of Difference				0.55	
Standard Error of Prediction				0.77	

Table 4 contains the statistical data from the mill raw juice, the diffuser raw juice and the final molasses calibrations. All calibrations were calculated using the wavelength range of 1100-2350 nm, in increments of 4 for a total of 311 wavelengths. The default software scatter correction was applied and the first derivative was used for the modified partial least square (PLS) calibrations.

Table 4 Statistical data for diffuser and mill juice and final molasses calibrations

Sample	Constituent	n	mean	SEC	R^2	SECV	1-VR
Diffuser	Brix	414	13.51	0.23	0.97	0.23	0.97
	pol	408	11.33	0.13	0.99	0.15	0.98
Mill	Brix	466	14.00	0.15	0.97	0.16	0.97
	pol	469	11.74	0.14	0.97	0.18	0.95
Final Molasses	Brix	361	40.81	0.35	0.93	0.37	0.92
	pol	358	13.21	0.41	0.72	0.44	0.66

Figure 1 compares the NIR predicted data and the lab reference data used to calculate the calibration for Brix in diffuser raw juice. Figure 2 is a residual plot for the pol data from the calibration set for mill raw juice. Both Figures illustrate a good calibration based on excellent data. Molasses pol data illustrated in the scatter plot in Figure 3 does not look as impressive. The statistical data in Table 4 paints a similar picture for molasses. Observing the plot on Figure 3, one can see that the data points are segregated into lines. This is due to a limitation with the lab pol data. All of the samples of higher Brix than syrup were diluted 1:1 and calibrated against the lab data for the 1:1 dilution. These samples are analyzed for pol using 26 g of the 1:1 diluted sample and diluting that further in a 200 ml volumetric flask. Limitations of the instrumentation, such as a single decimal place polarimeter, are amplified by these dilutions.

Tables 5 and 6 contain statistical data about other calibrations conducted including individual sample streams and combined calibrations of similar samples types. Statistical data on the juice calibrations in Table 5 demonstrate that all the juice types are suitable for NIR analyses. Also in Table 5 is data on a calibration of all the juices combined, which indicates a good possibility for a single juice calibration.

Samples of Syrup were diluted 2:1 for scanning and subsequent analysis by reference methods. The SEC and SECV values for the syrup calibration are higher than expected even thought the R^2 and 1-VR values are respectable. A massecuite exhibits the opposite; the SEC and SECV values are fairly low but the R^2 and 1-VR are low. This is due in large part to the lack of range in the data. Combining the massecuites, molasses and magma into one calibration set, helped to solve this issue with the A massecuites and prove to be a suitable calibration for most of the data sets.

Figure 1. Diffuser Juice Brix; Predicted vs. Reference

Slope: 1.005   RSQ: 0.985
SEP: 0.191 BIAS: -0.001 SEP(C): 0.191
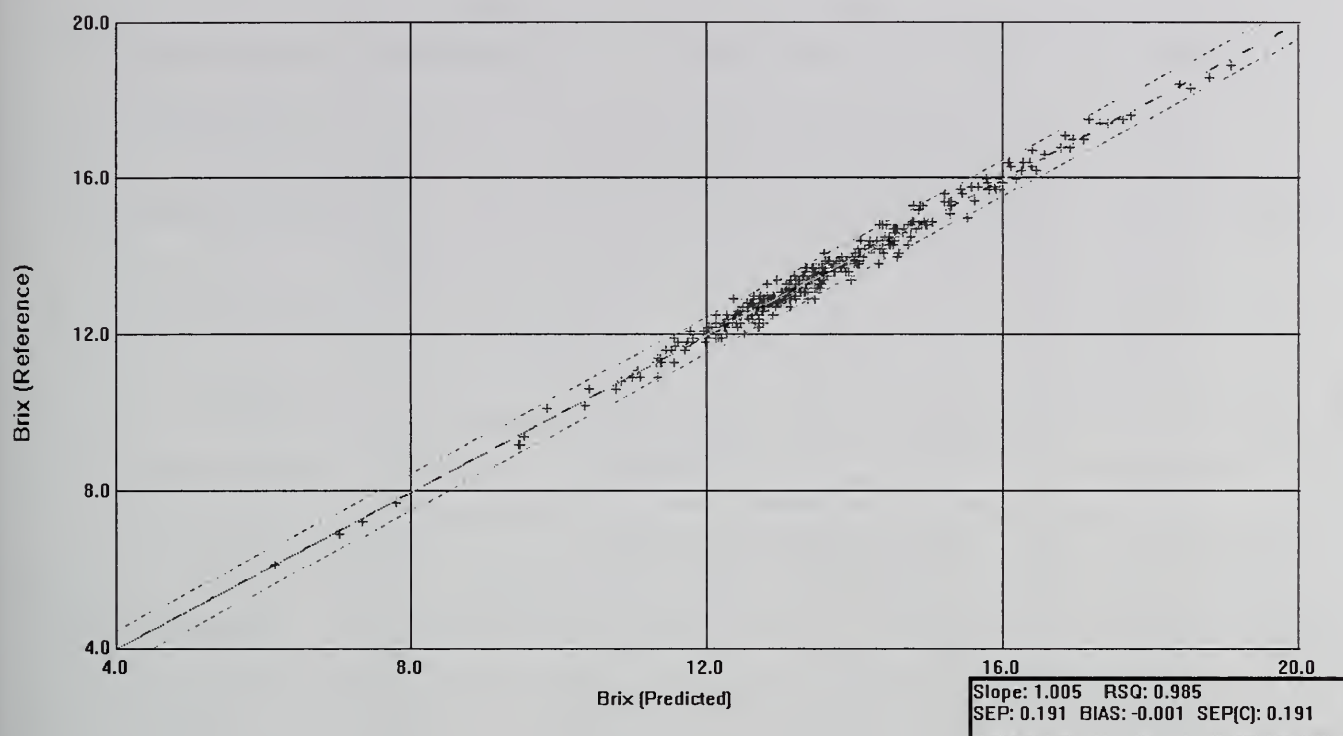
Figure 2. Mill Juice Residual Plot
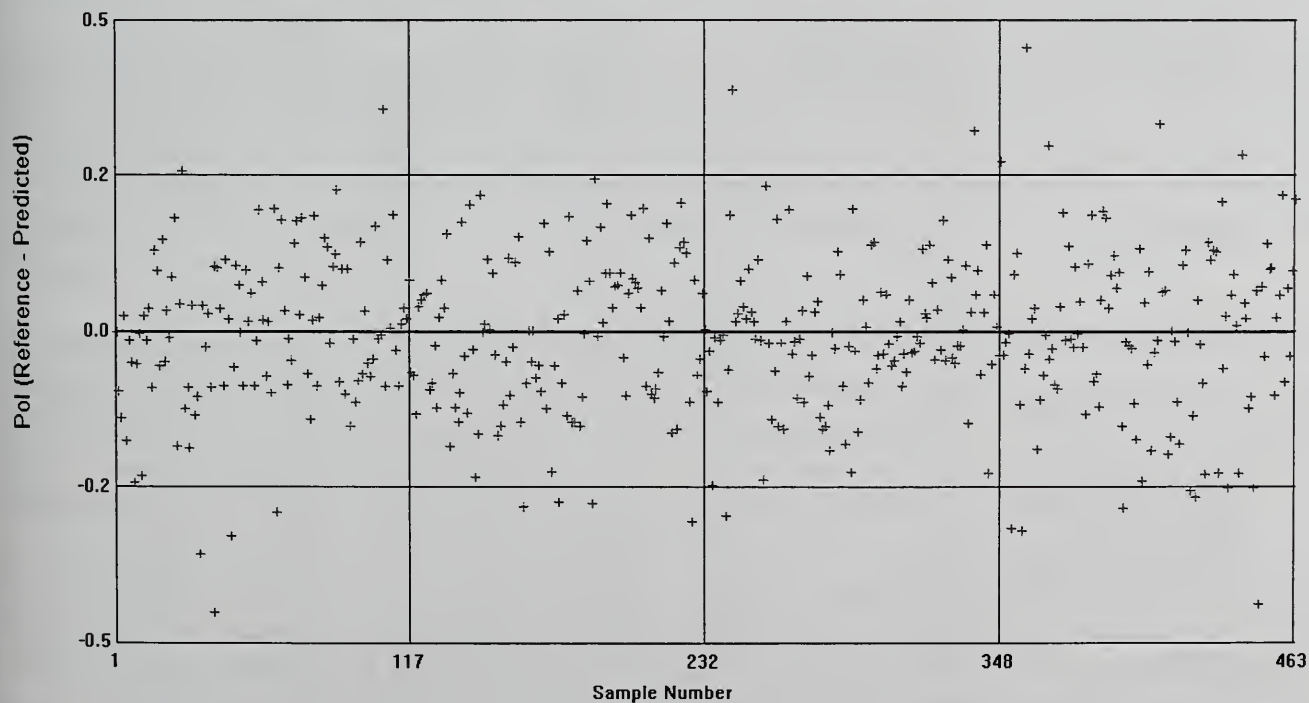
Figure 3. Final Molasses Pol; Predicted vs. Reference

Slope: 0.984   RSQ: 0.719
SEP: 0.398 BIAS: 0.001 SEP(C): 0.399
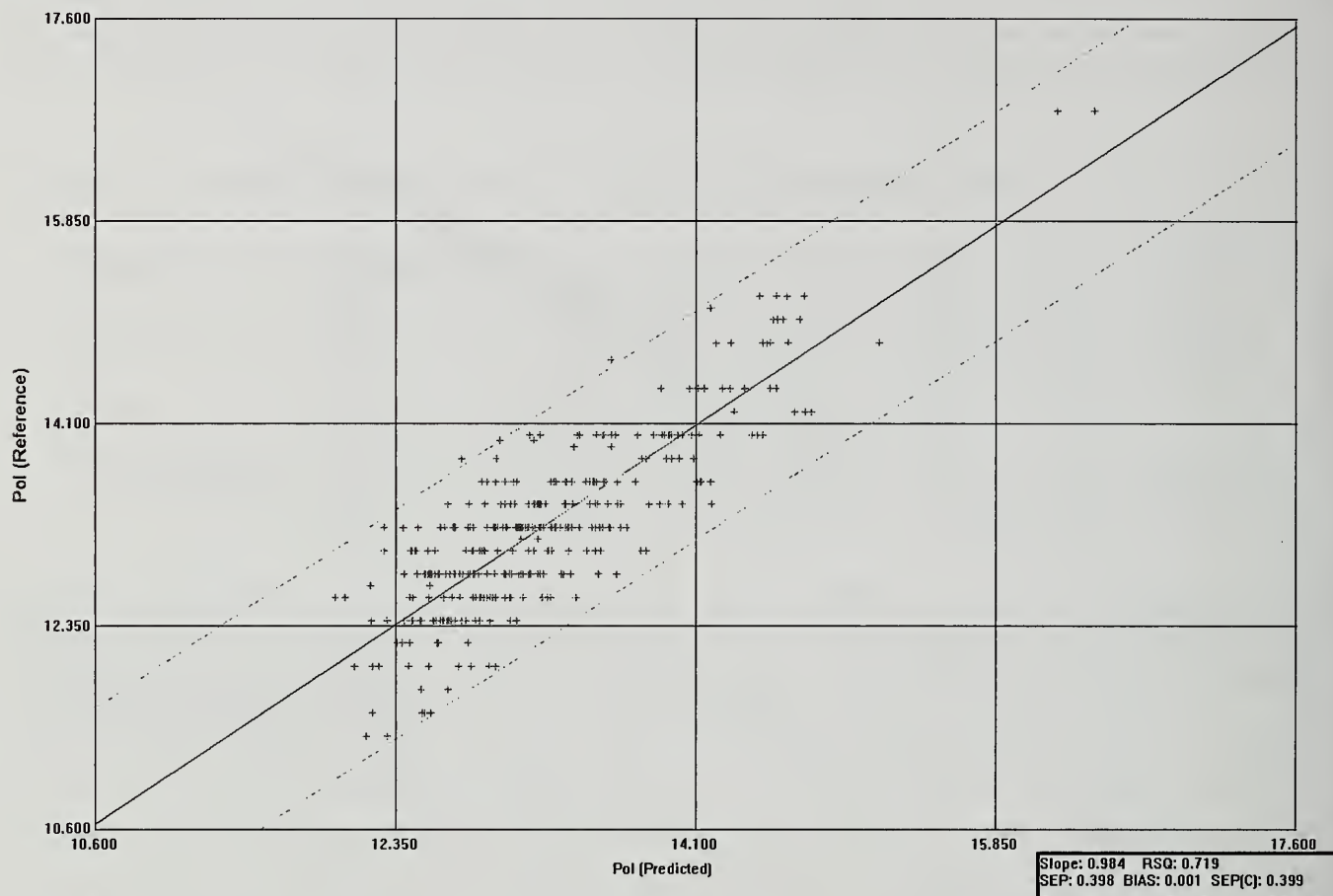
Table 5. Statistical Data of Juice and Syrup Calibrations

Equation for	Constituent	n	Mean	SEC	R^2	SECV	1-VR
Crusher	Brix	314	15.40	0.20	0.91	0.20	0.90
	pol	310	13.00	0.12	0.96	0.16	0.93
Mill Last Roll	Brix	312	9.74	0.14	0.99	0.15	0.99
	pol	310	7.65	0.13	0.99	0.15	0.99
Mill Clarifier	Brix	435	13.90	0.14	0.98	0.16	0.98
	pol	440	11.68	0.12	0.98	0.14	0.97
Diffuser Last Roll	Brix	291	3.07	0.14	0.98	0.14	0.98
	pol	285	2.29	0.08	0.99	0.10	0.99
Diffuser Clarifier	Brix	557	13.61	0.14	0.99	0.15	0.99
	pol	564	11.23	0.10	0.99	0.12	0.99
Pre-Evaporator Diffuser Juice	Brix	301	20.11	0.28	0.99	0.29	0.99
	pol	295	16.89	0.31	0.98	0.34	0.97
Combined Juice	Brix	3121	13.05	0.21	1.00	0.22	1.00
	pol	3167	10.84	0.19	1.00	0.20	1.00
Syrup	Brix	566	31.13	0.52	0.97	0.562	0.96
	pol	564	26.37	0.56	0.94	0.581	0.94

Table 6. Statistical Data for Massecuite, Molasses and Magma Calibrations

Equation for	Constituent	n	Mean	SEC	R^2	SECV	1-VR
A-Massecuite	Brix	2427	45.69	0.33	0.54	0.33	0.53
	pol	2440	38.80	0.49	0.75	0.50	0.74
B-Massecuite	Brix	767	47.09	0.39	0.52	0.39	0.51
	pol	768	33.40	0.40	0.91	0.42	0.90
C-Massecuite	Brix	767	47.63	0.45	0.63	0.47	0.61
	pol	765	26.17	0.57	0.87	0.61	0.86
A-Molasses	Brix	461	37.35	0.39	0.95	0.41	0.94
	pol	459	25.23	0.35	0.96	0.37	0.95
B-Molasses	Brix	460	37.95	0.31	0.96	0.33	0.96
	pol	477	17.97	0.40	0.93	0.44	0.92
B-Magma	Brix	518	43.59	0.34	0.94	0.35	0.94
	pol	525	39.52	0.50	0.91	0.52	0.91
C-Magma	Brix	542	44.90	0.30	0.86	0.32	0.84
	pol	542	38.10	0.54	0.85	0.57	0.83
*ALL	Brix	6444	44.36	0.42	0.98	0.42	0.98
	pol	6352	32.59	0.51	1.00	0.51	1.00
All Molasses	Brix	1309	38.58	0.37	0.97	0.38	0.97
	pol	1305	19.26	0.39	0.99	0.41	0.99

*All massecuites, molasses, and magmas

Conclusions and Recommendations

NIR worked well on all the juice samples as has been demonstrated in other factories around the world. Higher Brix samples are more challenging, but as illustrated in the data from the final molasses lab error test, the NIR is capable of acceptable performance. The laboratory data on the higher Brix samples will never obtain the accuracy and precision as seen in the juice sample due to dilution. However, improvements can be made and should be in order to obtain the best data for factory control.

Combination of data set for the simplification and/or increase data range for improved accuracy is possible. The benefits and drawbacks will have to be determined as these calibrations are used in the factory. Some sample types will work better together than others and several combinations need to be evaluated. The simplification of the calibration process by decreasing the number of calibrations must be balanced against the accuracy obtained by the combined calibrations

Personnel at the Enterprise Factory are gaining confidence in the NIR spectrometer, but are not ready to fully rely on it for factory control. The NIR instrument met the requirement of the 2004 season project plan and has preformed as expected. Calibration sets are getting large enough to provide more reliable data but will continue to be updated with outlier samples.

Certainly no ground breaking discoveries were revealed in this study. However, the viability of NIR in a Louisiana sugar factory for factory control was verified. Louisiana does pose issues that many other sugar growing regions do not; due to it sub topical climate and variable ambient conditions.

As the Enterprise factory relies more on the NIR and starts to reduce the staff in the control lab and the chemicals and supplies used, an evaluation of the actual savings can be calculated. The value of rapid accurate result will be harder to calculate, but should be considered.

Aknowledgements

The American Sugar Cane League
The Louisiana Board of Regents
Enterprise Factory for their assistance
Lee Madsen II of ASI
Shirley Anderson and Howard Eubanks of Foss North America

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Appendix A

3 June 2004

<u>A.   Beverage Module Standardization</u>

Step 1   Preparation

For best results, the instruments to be standardized should be located side by side.

Each instrument should have it's own PC with the software (ISIscan) parameters set up identically.

Step 2   Pathlength Adjustments

Set the pathlength on the instrument to 1mm +/- 0.02. The pathlength equation must be used to determine the pathlength.

Step 3   Create a standardization file.

<u>Determine the median sample</u>.   Scan a group of typical molasses samples. These samples should be collected in a .nir file.   Create a score plot to determine the sample which is closes to the mean.   This sample will be scanned in both instruments for standardization purposes.

<u>Create a master.nir file</u>. Scan the sample identified above 5 times in the master instrument.   This file will contain 5 scans. Export from ISIscan to WinISI and average these scans, save the average spectra in a file called master.nir.

To average a set of spectra, (WinISI) go to graphics mode to plot all spectra. Select Options, Average, this will create an average spectrum that can be seen in the spectra plot.   Then go again to Options, Save Selected Spectra, name this file master.nir. This file will contain all of the original spectra plus the averaged one. Delete and purge all of the individual scans so only the average remains.

<u>Create a host.nir file</u>. Repeat the procedure above with the same sample in the host instrument. Call the averaged spectra file, host.nir.
For best results, alternate the scans between master and host instrument until 5 scans in each are obtained.

<u>Create .std file (WinISI)</u> Copy the host.nir file into to master instrument PC.   Use the master and host nir files to create a .std file. This file is used in the host instrument to standardize spectra (to make it look like it came from the master instrument) and to predict results from the host instrument.

To create a .std file (WinISI) go to Instruments, Standardization, NIRsystems Instruments, Create Standardization File. Enter the names of the host and master.nir file and .std file.   The instrument serial number

can be incorporated into the master and host nir files; example m1234.nir and h5678.nir

Step 4   Creating a "Rep" file.

A repeatability spectra file "Rep file" is useful to compensate for instrument and temperature variation.

Instrument variability - scan a set of 10 samples on both host and master instrument.   The host spectra are standardized using the std file made above. Number all 10 scans with the same sample number.

Temperature variability – scan a set of samples at various temperatures to compensate for spectral changes caused by temperature.   Samples can be scanned cold and several more times as they warmed to room temperature. This can be done on the master instrument or the host instrument. Number all of these samples with the same sample number.

All spectra are merged into a common nir file (WinISI). This file is used when making the equation.

Step 5   Create the calibration

The calibration is created from the spectra from the master instrument and the Rep file.

B   Implementing the equation with the host instrument

Create a Standardization Profile in ISIscan and import the standardization file.

The calibration equation is imported into the Prediction Model (ISIscan) for use with the host instrument.   The Standardization Profile is also identified in the Prediction Model.

C   Determining Errors

Lab vs. NIR Error The lab versus nir error is determined for each constituent according to the attached procedure.   Typically the lab vs. nir error is less that 1.5 times the lab error.

FOSS

Lab vs. NIR

How to Evaluate Your Laboratory

An important part of evaluating the utility and performance of NIR is determining the error in the reference method. For a complete evaluation, it is necessary to determine both how precise each of the methods are as well as determining how accurately NIR predicts the reference values. A typical laboratory evaluation is described here.

Sample Split and Encoding

Laboratory

NIR
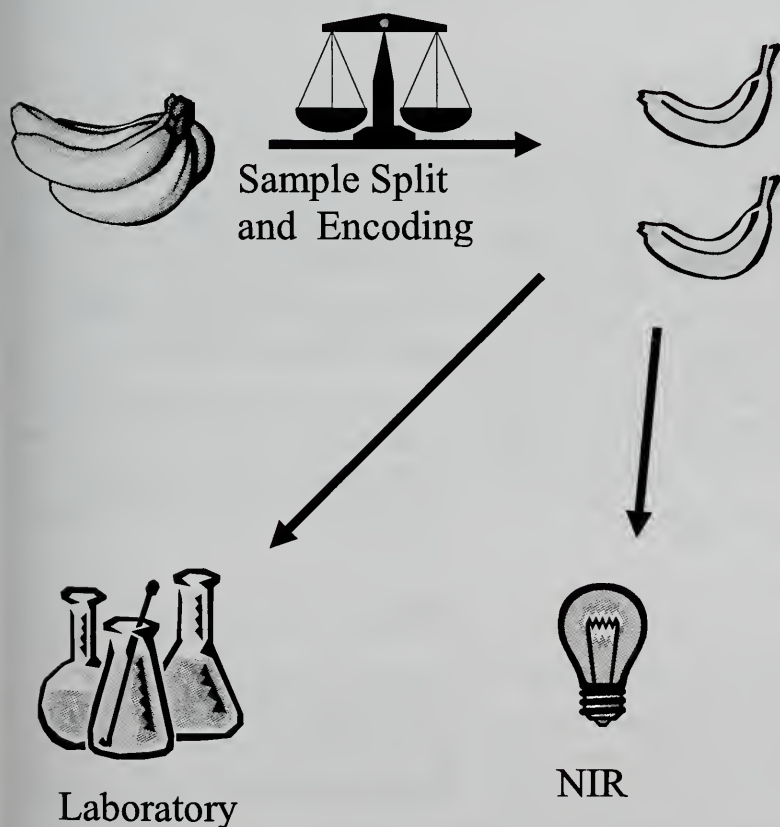
Step 1.

In the first step of the evaluation, 10 or more samples are split into blind duplicates and encoded with new sample numbers. It is very important that the sample split be done as equally as possible and that the original identity of the samples be hidden during the subsequent analysis.

Step 2.

The samples are then analyzed according to the Standard Operating Procedures in the laboratory and in the NIR.

FOSS

TB-301 2/98
Table 1.

Sample	Rep 1	Rep 2	D	(D)²
1	58.75	59.17	-0.42	0.18
2	59.80	60.31	-0.51	0.26
3	58.43	58.55	-0.12	0.01
4	59.39	59.52	-0.13	0.02
5	57.60	57.61	-0.01	0.00
6	57.90	58.15	-0.25	0.06
7	59.65	59.45	0.20	0.04
8	59.99	59.71	0.28	0.08
9	58.01	58.08	-0.07	0.01
10	59.33	59.36	-0.03	0.00
			Sum	0.655

Standard Error of Difference

$$\sqrt{\frac{\sum (D)^2}{N}} = \sqrt{\frac{0.655}{10}} = \mathbf{0.26}$$

Step 3.

Data from the laboratory are collected and the difference (D) between the blind duplicates are calculated. These differences are then squared and summed as shown in Table 1.

Step 4.

Standard error of differences (SED) is calculated according to the formula shown.

Table 2.

Sample	Lab Sub 1	NIR Sub 1	Diff	(Diff)²	Lab Sub 2	NIR Sub 2	Diff	(Diff)²
1	58.75	59.26	-0.51	0.26	59.17	59.10	0.07	0.00
2	59.80	59.95	-0.15	0.02	60.31	60.05	0.26	0.07
3	58.43	57.96	0.47	0.22	58.55	58.09	0.46	0.21
4	59.39	58.90	0.49	0.24	59.52	58.93	0.59	0.35
5	57.60	57.69	-0.09	0.01	57.61	57.78	-0.17	0.03
6	57.90	57.59	0.31	0.10	58.15	57.87	0.28	0.08
7	58.65	58.42	0.23	0.05	58.45	58.48	-0.03	0.00
8	59.99	59.83	0.16	0.03	59.71	59.98	-0.27	0.07
9	58.01	57.56	0.45	0.20	58.08	57.60	0.48	0.23
10	59.33	59.72	-0.39	0.15	59.36	59.76	-0.40	0.16
			Sum	1.28			Sum	1.20

Total　　2.48

Step 5.

To calculate the Standard Error of Prediction (SEP), enter the LAB and NIR values into a table and determine the differences as listed in Table 2.

Standard Error of Prediction

$$\sqrt{\frac{\sum (x_i - y_i)^2}{N}} = \sqrt{\frac{2.48}{20}} = \mathbf{0.35}$$

Step 6.

Calculate the SEP according to the formula listed.

In the example listed above, the SED of the laboratory was 0.26% protein and a similar calculation on the NIR values gave a SED of 0.13%.

The standard error of prediction (SEP) between the NIRSystems Feed Analyzer and the laboratory was 0.35

Improvement in the Circulating Efficiency By Radial Flow Impellers in Vacuum Pans Operated in Horizontal Cascade Crystallizers

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Abstract

For decades the impellers in vacuum pans have been installed within the central draft tube of the calandria. The axial flow impellers - so called "Kaplan type" - are nowadays the standard impellers in vacuum pans. In comparison with this standard type, the performance of radial flow impellers installed below the heating chambers of two vacuum pans at Goslawice Sugar Factory was investigated during the 2004 campaign. The two vacuum pans were operated continuously as the 3rd as well as the 2nd stage of B (raw-sugar) and C (afterproduct) cascade crystallizers. The investigations included running each impeller at three different values of the rotational speed and measuring the power consumption of the agitator drive while determining the heat transfer coefficient in the respective pan. In a similar manner, the performance of standard Kaplan Impellers installed in the central tubes of the heating chambers of both pans was investigated.

In relation to Kaplan impellers, the radial flow impellers made it possible to attain markedly higher heat transfer coefficients at lower values of the rotational speed. By comparing their power consumption at similar levels of the heat-transfer intensity, a comparison between the two impeller types with respect to the circulating efficiency was also made. In B3 vacuum pan, the circulating efficiency of the radial flow impeller was higher than that of the Kaplan impeller by a factor of 2.8. In C2 vacuum pan, the corresponding factor was 2.6.

Introduction

A horizontal cascade crystallizer was put into operation for the first time in 1981 in the Lage Sugar Factory in Germany. It consisted of five vacuum pans connected in series to crystallize after product sugar. A number of horizontal cascade crystallizers employing the same structural

principle were later installed at other locations in Germany and other countries. Two of these locations deserve to be mentioned because ground-breaking engineering solutions were applied there:

- Brühl Sugar Factory (late 1980s, four pans to crystallize white sugar);

- St. Michaelisdonn Sugar Factory (since 1994, three pans to crystallize after product).

Practical experience supported by theoretical considerations has proved that vacuum pans operated in a cascade crystallizer must be equipped with agitators. This is motivated by the need for homogenizing magma to achieve a high crystal quality, as well as the need for improving heat transfer to facilitate cascade heating with a small temperature difference between heating steam and magma.

For historical reasons, most stirrers currently applied in vacuum pans, including those operated in cascade crystallizers, are equipped with Kaplan-type impellers. Placed in the central draft - tube where baffles are also installed, such impellers exert an axial pumping action on the magma stream flowing in a downward direction. Flowing out of the draft - tube and against the cone-shaped central part of the bottom plate of the pan, the magma then changes its flow direction to radially outwards.

A new idea for augmenting the magma flow came from Dr. Klaus Kipke at the beginning of 1990s. Instead of axial pumping action in the draft - tube, he proposed radial pumping of the magma in the space between tube outlet and bottom plate of the pan. In order to implement this idea, the agitator should be equipped with a special impeller rotor placed below the tube outlet, and baffles should be installed in the draft - tube close to its outlet. After carrying out laboratory tests in 1991, the new stirring technique was implemented by Stelzer Rührtechnik GmbH for tests in Aarberg Sugar Factory in Switzerland in 1992 (Bruhns, *et al.*, 1993) and the industrial application in Gross-Munzel Sugar Factory in Germany since 1995. For measuring the heat transfer coefficients the pan in Aarberg was operated in a way that simulated the operation of a continuous pan and the pan in Groß Munzel was operated in a batch mode.

The present paper is devoted to full-scale industrial tests carried out in Gosławice Sugar Factory in Poland in 2004, of two different impeller types. These were optimized for application in two vacuum pans continuously operated as parts of horizontal cascade crystallizers, respectively:

- Unit B3 installed in the third stage of four-unit cascade for B (raw) sugar;

- Unit C2 installed in the second stage of three-unit cascade for C sugar (after-product).

For both units, the tests were so organized to facilitate comparisons of the efficiency of radial flow impellers with that of Kaplan-type impeller. It should be stressed that Kaplan-type impellers were also optimized for the units in question. The layout of impellers in Unit B3 is shown in Figure 1, and characteristic data of both vacuum pans and their impellers are given in Table 1.
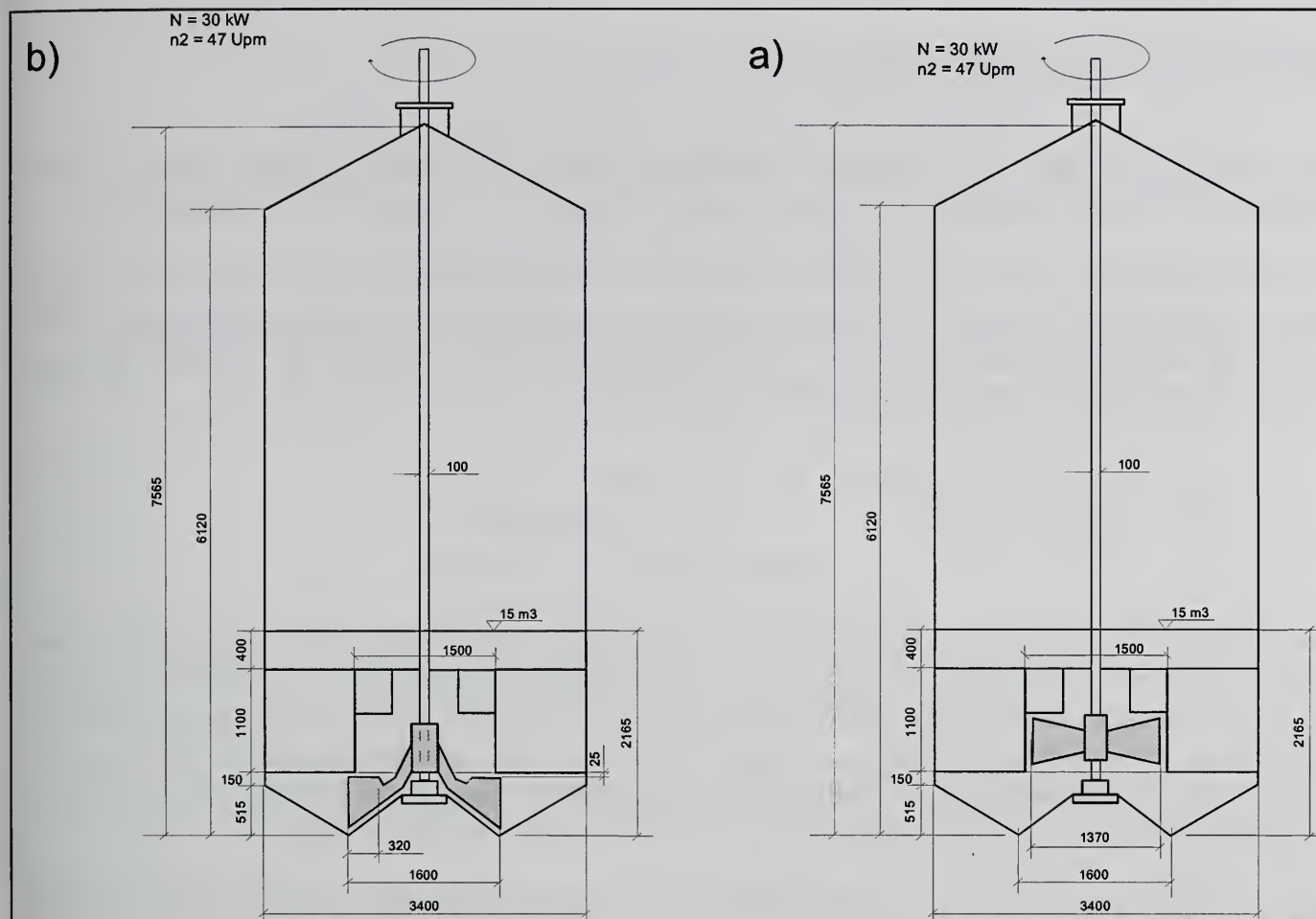
Figure 1.   Schematic cross-section of vacuum pan B3:
a) equipped with Kaplan-type impeller,
b) equipped with radial flow impeller.

Table 1. Characteristics of vacuum pans and impellers tested in Gosławice Sugar Factory.

		Vacuum pan	
		B3	**C2**
Rating for batch operation,	t	40	50
Area of heating surface,	m^2	200	260
Vessel diameter,	m	3.40	3.75
Diameter of Kaplan impeller,	m	1.37	1.40
Diameter of radial flow impeller	m	1.60	1.70
Diameter of draft- tube,	m	1.50	1.58
Tube length.	m	1.10	1.15
Tube diameter	m	0.10	0.10

Method of Measurement

For each unit, the tests were aimed at determining the heat transfer coefficient under a clean condition of the heating surface while also recording the power consumed by the agitator drive.

The determination of the heat transfer coefficient in a crystallization unit is based on its heat balance. Neglecting heat loss to the environment, heat supplied by the heating steam equals heat transferred across the heating surface. This relationship makes it possible to calculate the heat transfer coefficient as (symbols and indices see below):

$$k = \frac{\dot{m}_{Co}(h_{St} - h_{Co})}{A(T_{St} - T_{Ma})} \tag{1}$$

The mass flow of steam is determined from volumetric measurement of the condensate flow using a cylindrical test vessel (Volume 1 m³) equipped with a graduated level indicator (the test vessel can be connected to either of the crystallization Units B3 or C2). The values of steam temperature, and specific enthalpy of steam and condensate, are determined on the basis of measurement of steam pressure. The mean temperature of magma is determined from direct temperature measurements at two points above and below the heating chamber.

Prior to initiating the tests of a crystallization unit, it is disconnected from the cascade, its heating surface is cleaned and the impeller rotor is installed together with guide vanes in a suitable arrangement. The unit is then connected to the cascade and returned to normal operation. Before collecting a complete set of measurement data, the required value of the rotational speed of the impeller is set using frequency controls of the agitator and the condensate stream leaving the heating chamber is directed to the test vessel. The measurement is started by simultaneously recording the start time and initial condensate level in the vessel, and finished by recording the end time and the final condensate level. During the measurement period, readings of current consumed by the agitator drive are taken from the display of the frequency converter. Magma and Nutsch samples are also taken for laboratory determination of dry substance content, purity and crystal content.

Other data are automatically acquired from standard factory instrumentation and stored in the computer memory. In addition to the variables necessary for determining the heat transfer coefficient, the data set includes other variables (dry substance content of magma, magma level, mass flow of syrup supplied to the unit, mass flow of seed magma supplied to the cascade) making it possible to check whether or not stable operating conditions have been maintained throughout the measurement period.

After finishing the measurement, the data are processed according to a procedure programmed in MathCad software. The procedure, schematically shown in Figure 2, includes filtering and time-averaging of the values of variables appearing in equation (1). The values of thermodynamic functions are calculated using a software module called "MathCad steam extension pack".

Input of raw measurement data from:
- automatic measurements of process parameters
- volumetric measurement of condensate flow

↓

Calculation of condensate mass flow

↓

Filtering and time-averaging of data from automatic measurements
incl. Steam pressure and magma temperature at two points

↓

Calculation of thermodynamic functions:
- steam temperature
- specific enthalpy of steam
- specific enthalpy of condensate

↓

Calculation of mean magma temperature

↓

Calculation of heat transfer coefficient from fomula (1)

Figure 2. Flow diagram of the processing of experimental data.

Results

As there was at least a period of 24 hours between the tests of different impellers in the same crystallization unit, it turned out to be impossible to reproduce the operating conditions exactly - especially the state of the magma - from day to day. It should therefore be remembered that changes in magma temperature, magma purity, DS content and crystal content, affecting the physical properties of magma (mainly its density and dynamic viscosity), may influence the value of the heat transfer coefficient. Additional influence could be expected from bigger changes in temperature difference between heating steam and magma, leading to a change in the mechanism of heat transfer. Changes in temperature difference of that extent were not observed. Furthermore, changing mass flow of seed magma supplied to the crystallization cascade, as well as that of syrup fed to the crystallization unit for control purposes, affect magma flow conditions heat transfer and thus also influence the heat transfer coefficient.

It follows from the inspection of measurement data that generally, variations in magma temperature and DS content were negligibly small. More important were variations in the crystal content as indicated in Table 2.

Table 2. Ranges of magma parameters recorded during the test period.

Unit	Range of magma DS content %	Range of magma temperature °C	Range of crystal content, %			
			Kaplan type impeller		Radial flow impeller	
			General	Most measurements	General	Most measurements
B3	91.0-92.4	69.0-71.0	40-50	46-50	40-42	40-42
C2	91.1-94.1	74.4-77.0	29-42	35-42	29-42	29-32

More information on the conditions for measurement in Unit B3 is given in Figure 3, and that for Unit C2 is given in Figure 4.
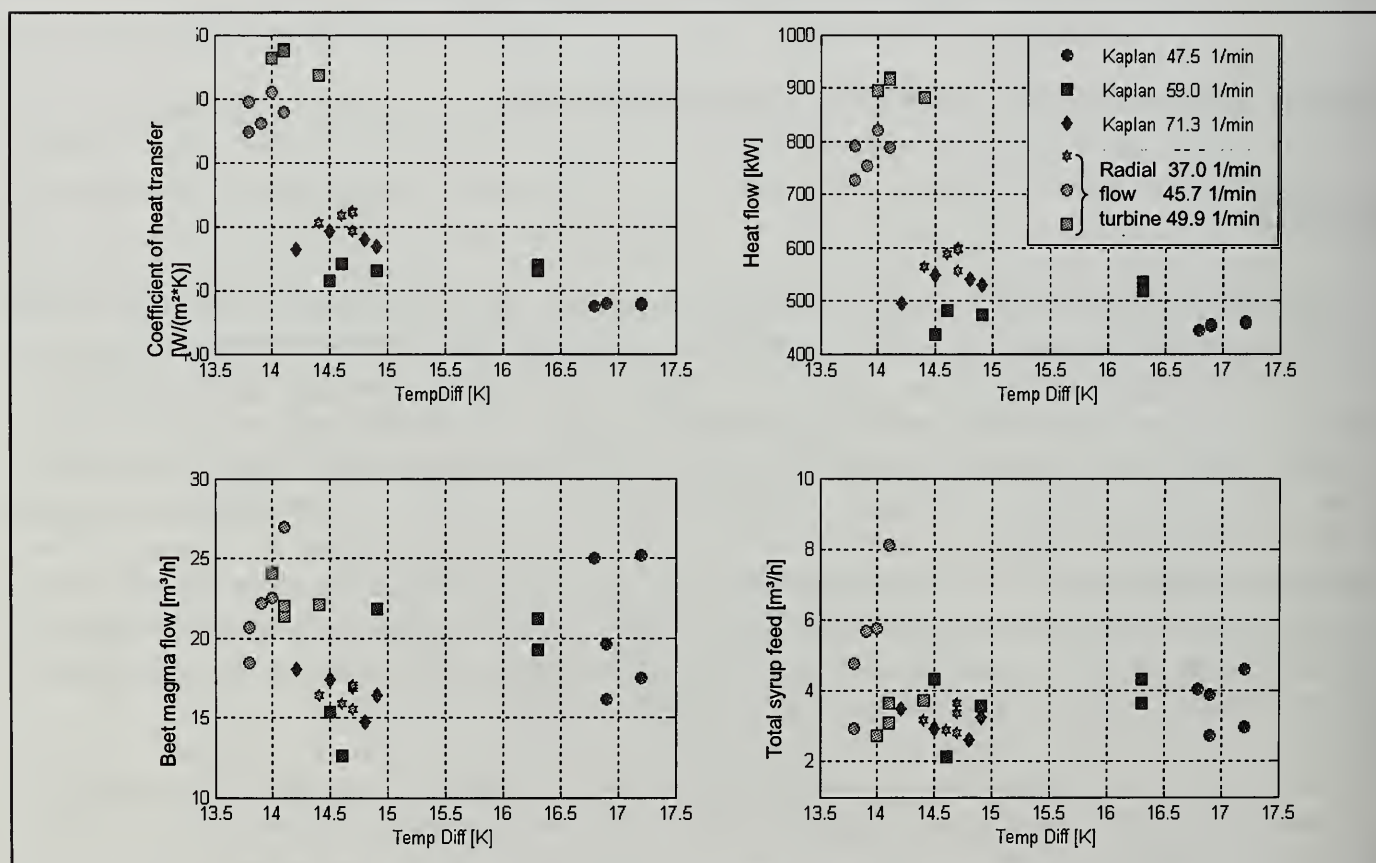
Figure 3. Crystallization Unit B3: Heat transfer coefficient, heat flux, mass flow of magma atcascade inlet and mass flow of syrup supplied to the vacuum pan versus temperature difference between heating steam and magma.
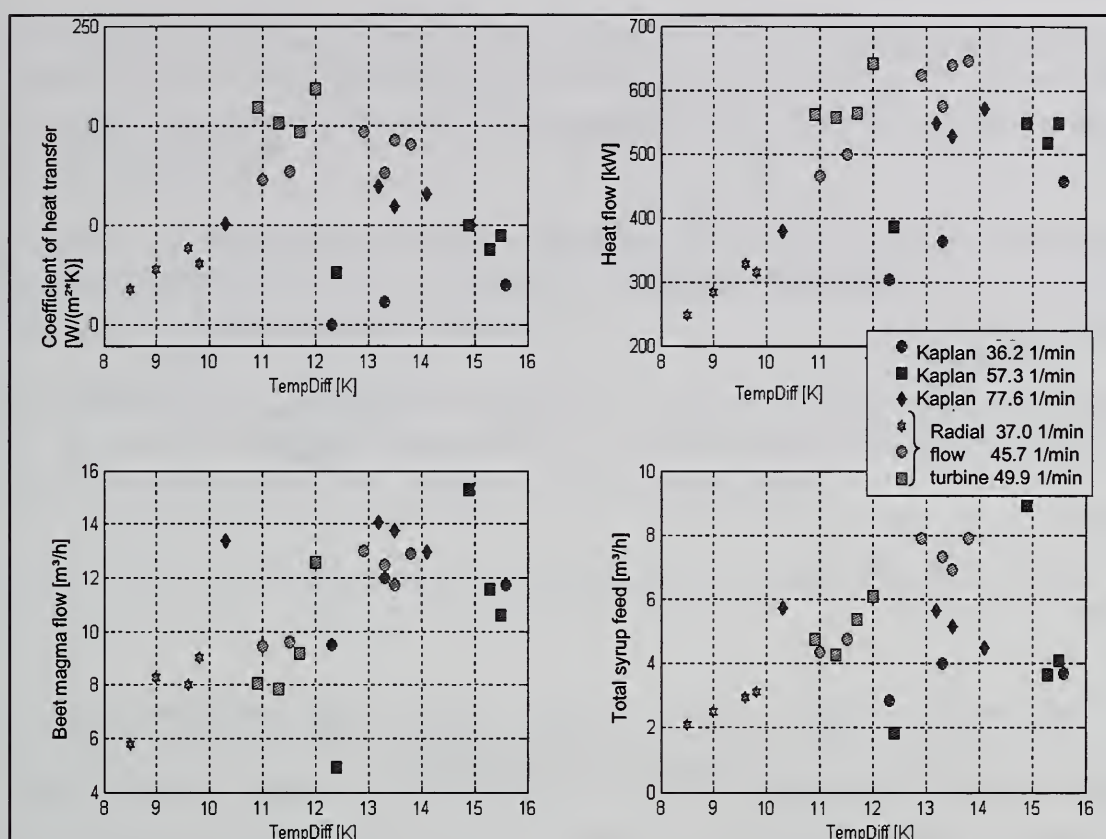
Figure 4. Crystallization Unit C2: Heat transfer coefficient, heat flux, mass flow of magma at cascade inlet and mass flow of syrup supplied to the vacuum pan versus temperature difference between heating steam and magma.
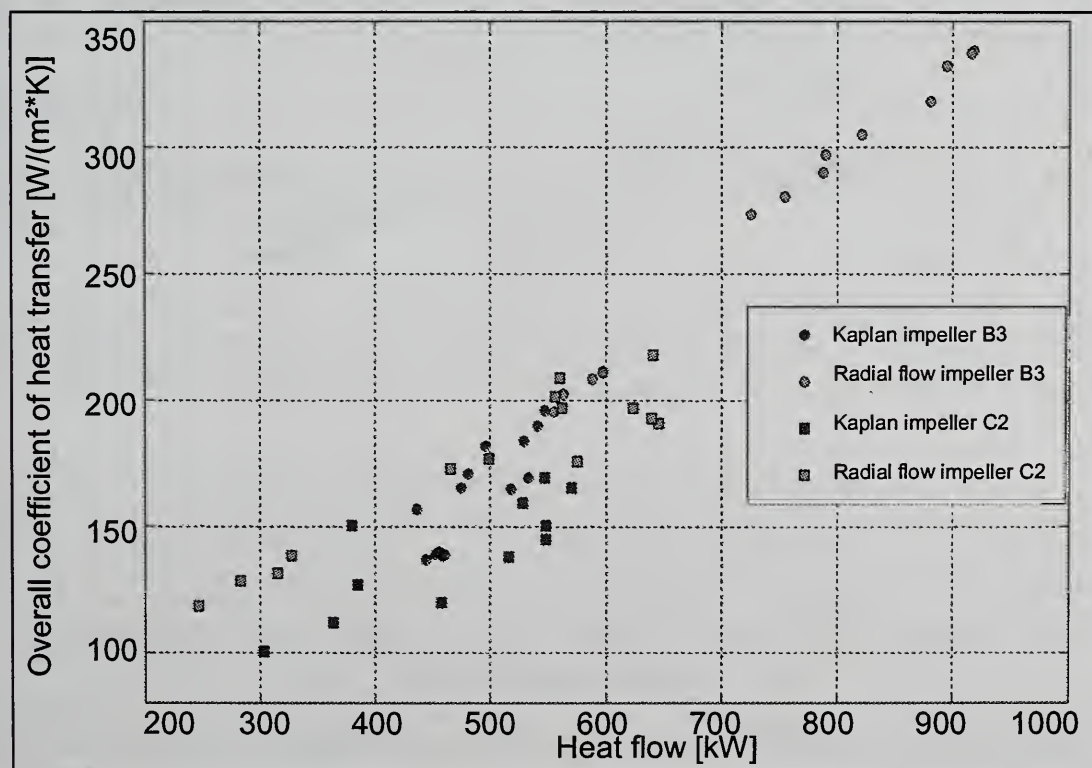
Figure 5. Crystallization Units B3 and C2: Heat transfer coefficient as a function of heat flow across heating surface.
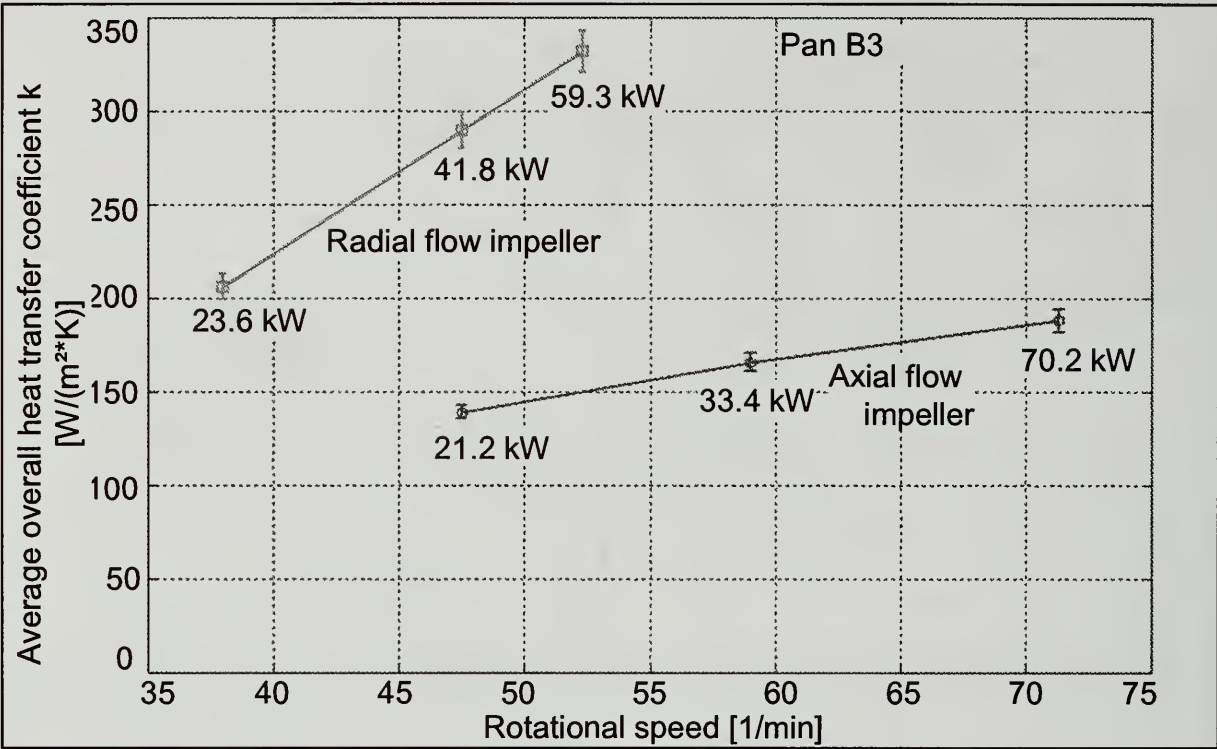
Figure 6. Crystallization Unit B3: Heat transfer coefficient (and power consumption of agitator drive) versus rotational speed of the impeller.
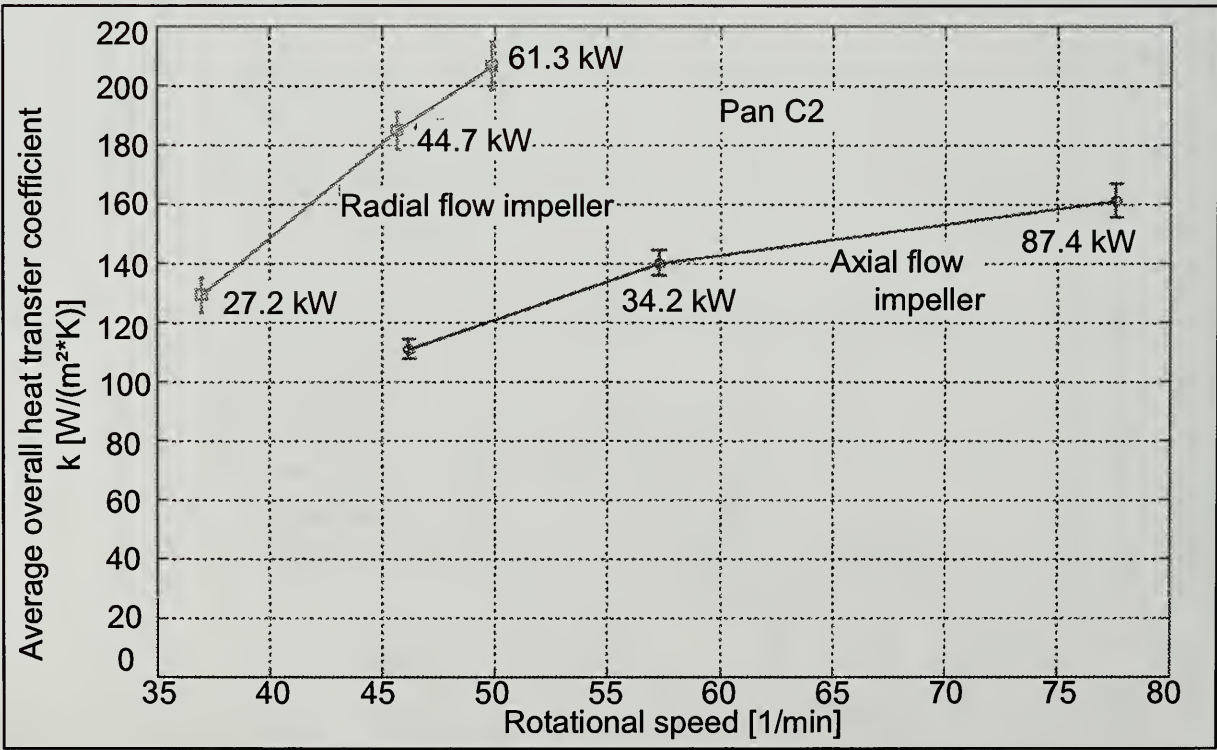
Figure 7. Crystallization Unit C2: Heat transfer coefficient (and power consumption of agitator) versus rotational speed of the impeller.

A synthetic view of the influence of operating conditions on the measurement results is given in Figure 5 where operating conditions are represented by the values of heat flux across the heating surface. As might be expected, the relationship between the heat transfer coefficient and the heat flux is approximately linear.

In Figures 6 and 7, the average heat transfer coefficients are shown as functions of the impeller rotational speeds for Units B3 and C2, respectively. Average values of the power consumption of the respective agitator drives are also indicated.

In both crystallization units, radial flow impellers are characterized by sharper increases in k values with increasing rotational speeds. If both impeller types are operated at the same rotational speed, then the radial flow impeller consumes more power.

Comparison Between Kaplan-Type Impeller and Radial Flow Impeller

The results of measurements carried out at Goslawice Sugar Factory prove that - for the conditions found in Goslawice - the radial flow impeller makes it possible to attain a heat transfer intensity higher than that characteristic for the Kaplan-type impeller (axial flow impeller). It has to be considered, that this comparison has been made in pans which have been optimized by technical evolution and not by scientific methods. There is a great chance that by applying of fluid mechanics to the layout of the pans (dimensions and shape) with radial flow impellers the performance in terms of heat transfer can be improved much further than was seen in Goslawice. By applying the radial flow impellers in stirred continuous pans, a number of advantages can be attained.

Direct Advantages

After replacing the Kaplan-type impeller by the radial flow impeller, two extremely different courses of action can be envisaged:

A) Maintaining unchanged heat transfer intensity at a lower rotational speed. As a consequence, power consumption of the agitator is reduced (making it possible to improve crystal quality). This can be illustrated by sample data taken from Figures 6 and 7, and shown in Table 3.

Table 3. Power consumption of agitator drives in crystallization units.

Unit	Kaplan-type impeller		Radial flow impeller		Power reduction factor
	Heat transfer coefficient W/(m^{2}K)	Power consumption kW	Heat transfer coefficient W/(m^{2}K)	Power consumption kW	
B3	150	70.2	150	23.6	3.0
C2	160	87.4	160	34.0	2.6

B) *Intensifying heat transfer at a similar level of the rotational speed.* In general, a higher heat transfer intensity causes the evaporating capacity of the unit to increase. Assuming that an unchanged temperature difference between the heating steam and magma is maintained, sample data based on Figures 6 and 7 are shown in Table 4.

Table 4. Effect of heat transfer intensity on evaporation in crystallization units.

Unit	Kaplan-type impeller		Radial flow impeller		Evaporation increase factor
	Heat transfer coefficient $W/(m^2K)$	Power consumption kW	Heat transfer coefficient $W/(m^2K)$	Power consumption kW	
B3	140	47.5	290	47.5	2.1
C2	115	46.0	195	46.0	1.7

It can be added that between the two extremes A) and B) above, intermediate courses of action can be chosen to attain a trade-off between heat transfer intensification and power saving.

Indirect Advantages

When increasing the intensity of heat transfer across the heating surface at a constant magma temperature, the evaporating capacity of the crystallization unit can be stabilized by heating it with steam at a lower temperature (possibly, vapours extracted from another evaporator effect). If this is done for a group of crystallizers, then the heat economy can be improved leading to a saving in fuel consumption in the power house. The amount of fuel saved depends on the initial situation and the extent to which heat economy is changed, and is typically of the order of several percent of the initial fuel consumption. In addition, a lower steam temperature may result in less color build-up in crystallization thus contributing to improved sugar quality.

Concluding Remarks

It is advisable to compare the characteristics of radial flow impellers tested in Goslawice Sugar Factory with those of the impeller tested 12 years earlier in Aarberg. To make the comparison complete, one should also consider the impeller Reynolds number defined as:

$$Re_{Stir} = \frac{n \cdot d^2 \cdot \rho}{\eta} \qquad (2)$$

where: n – rotational speed in 1/s, d – characteristic diameter of the impeller rotor in m, ρ – magma density in kg/m^3, η – dynamic viscosity of magma in Pa·s.

One should also consider the relationship between the heat transfer coefficient and the stirring power, typically expressed as:

$$k \sim P^m \qquad\qquad (3)$$

where: P - power consumed by the agitator drive in kW, m – dimensionless constant characterizing the impeller and its operating conditions.

The key data on operating conditions and the main results of the tests of radial flow impellers performed in both sugar factories are shown in Table 5. To calculate Reynolds numbers, the magma density and dynamic viscosity were calculated as functions of magma temperature, DS content and crystal content using empirical equations given in the literature (Bubnik, *et al.*, 1995) or derived from the authors' own experimental data.

Table 5. Comparison of test data on radial flow impellers.

Sugar factory	Aarberg	Goslawice	
Vacuum pan rating for batch operation, tonne	70 (50 m^3)	40	50
Heating surface area, m^2	350	200	260
Vessel diameter, m	4.70	3.40	3.75
Mode of operation	Batch	Continuous in a cascade	
Product crystallized	White sugar	Raw sugar*	After product**
Temperature difference between heating steam and magma, K	Below 14	13.8-14.7	8.5-13.8
Crystal content in magma, %	45-50	40-42	29-42
Impeller diameter, m	1.90	1.60	1.70
Reynolds number Re$_R$	1000-1900	340-450	240-300
Power consumed by agitator drive, kW	8-19	23.6-59.3	27.2-61.3
Coefficient m	1/3.4<m<1/5.3	1/2.04	1/1.72
Maximum heat transfer coefficient, W/(m^{2}K)	340	338	210

*/ 3rd stage of a four-stage cascade; **/ 2nd stage of a three-stage cascade

References

Bruhns M., Schulz T., Sittel G, Wicke R. (1993), Neues Rührorgan für die Verdampfungs-kristallisation – Entwicklung im Technikum und Erprobung im Technischen Kristallisator. Zuckerindustrie 118, 853-858.

Bubnik Z., Kadlec P., Urban D., Bruhns M. (1995), Sugar Technologists Manual, Bartens, Berlin.

<u>Symbol</u>

A	heating surface	m^2
P	Power	kW
Re	Reynolds number	
T	Temperature	K (°C)
d	diameter of impeller	m
h	specific enthalpy	kJ/kgK
k	heat transfer coefficient	$\dfrac{W}{m^2K}$
n	rotational speed	s^{-1}
η	dynamic viscosity	Pa.s
ρ	density	kg/m^3
ṁ	mass flow	kg/s

<u>Indices</u>

Co	Condensate
Ma	Magma
St	Steam

The Potential of Cane Vinasse - Composition, Uses and Disposal

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Abstract

The production of ethanol from cane molasses has provided many benefits to the sugar industry. However, disposal of the vinasse that remains after distillation presents a major environmental challenge. From 10 to 15 tons of vinasse are produced for every ton of alcohol. This product is high in potassium, BOD and COD and lacks valuable constituents that other types of vinasse have, such as those from wine, beets and corn. Various uses for vinasse are discussed, and practices in China, India and Brazil are mentioned. The composition of vinasse is outlined. Some newer ideas on disposal include anaerobic digestion, bio-composting, and on-site incineration as an additional fuel source. Some work done with Colombian vinasse will be highlighted.

Definition of Vinasse

After ethanol is recovered from a fermentation broth, the dilute liquid that remains is known as vinasse. It has a total solids content of 2-4% when obtained from cane juice and 5-10% when obtained from molasses, the more common source. It represents a major environmental disposal problem because the volume is 10 to 15 times greater than the alcohol produced, and it has few compelling value-added constituents or uses. Vinasse is high in ash and high potency organic compounds, such as phenolics, organic acids, colorants, and is low in carbohydrates and protein.

Traditional uses of vinasse have been its disposal as waste to the environment (often without concentration), its use as a potassium fertilizer, and its use as an additive in cattle feed. Environmental restrictions have limited the former and consequently increased the use for the latter two purposes. The demand for these is limited, however, and a serious oversupply situation thus exists on the market. There is some interest in recovering components from vinasse.

Other names for this distillery by-product are vinasa (Spanish), stillage, distillery slops, distillery wastewater and spent wash.

Other Types of Distillery Wastewater Have Some Valuable By-Products
Fermentation of grapes (to make wine), corn, beet, citrus and whey also produce distillery wastewater. Most of the other types of vinasse, except for cane, are high in valuable by-products or high in nutritional content for animal feed. This has made it difficult to develop economical uses for cane vinasse. For example, beet stillage has 16-34% protein, compared to cane with 3-8% protein. In France, concentrated beet vinasse is used as a fertilizer by the distilleries (Decloux, *et al.*, 2002). Beet vinasse is high in betaine, a valuable by-product, and glutamic acid. Wine vinasse is high in tartaric acid.

Use of Corn Stillage
Corn industry stillage is the source of highly nutritious livestock feed. The stillage is sent through a centrifuge that separates the coarse grain from the solubles. The solubles are concentrated to about 30% solids by evaporation, resulting in Condensed Distillers Solubles (CDS) or "syrup." The coarse grain and the syrup are dried together to produce Dried Distillers Grains with Solubles (DDGS), a high quality, nutritious livestock feed. The CO_2 released during fermentation is captured and sold for use in carbonating soft drinks and beverages and for the manufacture of dry ice.

The Problem: Stillage Volume - Some Environmental Considerations
The major environmental problem with cane vinasse is the huge volume produced. One liter of vinasse is equal to the domestic sewage from 1.43 people in Brazil. This means that every gallon (3.8 liters) of ethanol produced equals the pollution load of 40 to 70 people. For a distillery producing 200 t/day of ethanol, the stillage would be similar to the sewage of a town with one million people.

Composition of Cane Vinasse

The proximate analysis of cane vinasse was reported by Dowd, et al. (1994), shown in Table 1. Wei and Xu (2004) reported on typical Chinese vinasse composition, shown in Table 2.

Table 1. Proximate analysis of cane stillage% (Dowd, et al, 1994)

Moisture	89.64
Protein	2.92
Fiber	0.2
Fat	0.41
Ash	3.61
Carbohydrate	3.42
PH	4.4

Table 2. Typical composition of Chinese vinasse (Wei & Xu, 2004)

Parameters	Values
pH	3.8-5.0
Dry matter	9-12%
Carbohydrate content	1.9-3%
Ash content	2-5%
Organic compounds	6-9%
Phosphorus (as P_2O_5)	0.01-0.03%
Potassium (as K_2O)	0.4-2.5%
Nitrogen (as N_2O)	0.02-0.23%
Calcium (as CaO)	0.20-0.55%
Magnesium (as MgO)	0.1-0.36%
BOD	45,000 - 70,000 mg/L
COD	100,000 - 150,000 mg/L

Organic Acids in Vinasse

Molasses vinasse is generally higher in organic acids than cane juice vinasse. The most common acids are lactic, aconitic, glycolic and citric. Smaller amounts of oxalic, malic, succinic and quinic acid are also found. Table 3 shows values for vinasse from Guadeloupe, FWI. Dowd, *et al*, (1994) in one sample of cane vinasse, found 5.86 g/L glycerol, 1.56 g/L acetic acid, 7.74 g/L lactic acid, and 0.43 g/L inositol isomers. No free amino acids or sugars were found in his sample.

Table 3. Organic acids in vinasse (mg/L) (Celestine-Myrtil and Parfait, 1988)

Acid	Cane juice vinasse	Molasses vinasse
Oxalic	8-10	20-100
Lactic	1700	3700-7200
Aconitic	300-500	1000-1200
Glycolic	nd	930-1900
Citric	nd	300-2000

pH 4-5
BOD = 30,000-40,000

Nitrogen Content of Vinasse

Japanese investigators studying Brazilian vinasse reported 7.16% amino acids by weight of dried vinasse, following hydrolysis with 6N HCl. The major amino acids were aspartic acid (1.07%) and glutamic acid (1.58%). Others were threonine (0.41%), serine (0.57%), glycine (0.43%), alanine (0.53%), valine (0.40%) and leucine (0.46%). Other amino acids were present in trace quantities (Serikawa, et al, 1993).

Options for Vinasse Disposal

The huge volumes of vinasse that are produced are a major environmental challenge. Some combination of chemical and biological treatments is necessary for optimal disposal.

Options for disposal of vinasse include (1) lagoon treatment, popular with many distillers because it is cheap, but it is a lengthy process and smells terrible; (2) land application, again a cheap disposal, but it may changes soil properties, causing the soils to "clog" and harden, to become saline, change pH and smells bad; (3) anaerobic digestion; (4) concentration and burning in special boilers. The latter system is described as a zero effluent system with advantages over the other systems, although it is energy intensive because of the need to evaporate a lot of water.

Biological Applications

An innovative application was proposed in India, using vinasse as a control for aphids and whitefly infestations in cotton plants. The raw vinasse was sprayed on 45-day old cotton plants. Up to 85.9% of aphid (*Aphis gossypii*) population was decreased by the vinasse. Whitefly was also controlled to a lesser extent. Bollworm was controlled with a mixture of vinasse and the insecticide fenvalerate. Productivity of the cotton plants increased (Sundaramurthy, 1998). Similarly, application of vinasse supplemented with several microorganisms enhanced the growth and yield of pigeon pea and sorghum (Babu, et al., 1996)

Vinasse was used as a substrate for the fungus *P. chrysosporium* to produce protein. The composition of the mycelia was fairly constant regardless of the method of culture, producing 30.84-33.28% crude protein on a dry weight basis (Cardoso and Nicoli, 1981).

Pretreatment of Vinasse – Removing Color, Phenolics, COD, Potassium

Vinasse has a high organic load and antibacterial activity due to the presence of phenolic and colorant compounds. Removing these can help in the bioremediation of vinasse, and make it more amenable to subsequent anaerobic fermentation. Removal is accomplished by biological or chemical pre-treatment.

Biodegradation of color and phenolics has been carried out with several types of organisms: *Geotrichum candidum, Aspergillus terreus, Coriolus versicolor, Phanerochaete chyrsosporium* and *Mycelia sterilia. Coriolus versicolor* and *Geotrichum candidium* were able to decrease color, phenolics and COD significantly, making the vinasse better able to sustain anaerobic fermentation (Fitzgibbon, et al, 1995). As much as 77% of the COD was removed. *Aspergillus niger* produced about 80% decolorization of vinasse (Dhamankar, 2004). In another study, *Aspergillus tereus* and *Geotrichum candidum* were shown to effectively degrade phenols (Garcia-Garcia, et al, 2005).

Ideas for non-biological remediation have included decolorization with granular activated carbon (Serikawa, et al, 1993) and adsorption with tetramethylammonium bentonite (Buchler, 1989). Ozone treatment and treatment with hydrogen peroxide have also been proposed. Ozonation in acid media gave a selective elimination of phenolic compounds and provided a more readily biodegradable waste (Martin Santos, et al, 2003; Martin Santos, et al, 2005). Hydrogen peroxide in the presence of 0.06 M NaOH removed 98% of the color and 85% of the COD in 15 minutes (Dhamankar, et al, 1993). At this time, these expensive remediation methods are mainly of academic interest.

Potassium and sulfate are both high in cane vinasse and strategies for reducing these are also in discussion, especially for successful anaerobic digestion. Decloux, *et al.* (2002) explored the use of electrodialysis to reduce potassium levels in beet vinasse, a procedure which might also work for cane vinasse.

Examples of Disposal in Various Countries

Vinasse Use in Brazil
Vinasse is used in Brazil as a fertilizer to increase cane production and to reduce the use of industrial chemical fertilizers (Amorim, 2005). The use of vinasse alone or mixed with nitrogen was shown by CTC in a 7-year study to significantly improve sugarcane yield and to act as a soil fertility improver that promoted deep root development (Penatti, et al, 2005). Adding nitrogen to vinasse improved the performance over using vinasse alone. Brazil also makes use of anaerobic digestion to dispose of vinasse.

Vinasse Use in China
China has experimented with several ways to dispose of, or otherwise use, vinasse.

Lagooning: This entails simple storage of vinasse in large open, unlined lagoons for 1-2 months. Suspended particles precipitate and the BOD gradually diminishes. The final liquid material is used to irrigate land or released into waterways. This is the cheapest option for vinasse disposal, but associated problems include bad smell, it takes too long, run-off problems or over-flow in periods of heavy rain. Lagooning reduces COD about 60%.

Land application: Over time, the soil characteristics were changed and degraded; plant yields declined, possibly due to salination of the soil, and there are very bad odor problems. This is also an inexpensive option except that transporting large volumes of liquid vinasse is a challenge.

Organic complex fertilizer: The vinasse is concentrated and nutrients added for desired crops. The mixture is dried before application. It is called a "tailored mixture."

Zero-effluent technology: The vinasse is concentrated in order to burn it. According to the authors (Wei & Xu, 2004), this system is easier to operate than an anaerobic digestor and has lower operating costs. The technology uses 3-effect flash evaporation. There is a problem with scale deposition in the boilers and evaporators and corrosion of the pumps and pipes due to the acid nature of the material.

Granular vinasse fertilizer: A granular vinasse was developed that is supplemented with nitrogen from fungal biomass with filter cake and bagasse used as fillers. The material is applied as a fertilizer on cane fields and is claimed to increase cane tonnage and sucrose content with no ill effects on germination or soil quality (Jiang and Xu, 2004).

Vinasse Use in India

In India, distillery effluent is either subjected to biomethanation (anaerobic digestion) process to produce biogas, or mixed with press mud cake and made into compost. In 1999, there were 150 biogas plants and 50 compost units in India.

India Bio-Composting System: This low investment approach with a 3-4 year payback was recently reported (Patil, et al., 2004). In short, filter cake and vinasse are mixed with a special starter culture. Filter cake is heaped on the ground, with occasional pits dug in it. About 2% boiler ash is added. Vinasse mixed with a specially developed starter culture of microbes consisting of a mixture of bacteria, fungi and actinomycetes is sprayed onto the filter cake at a ratio of 3 parts vinasse to one part filter cake. The mixture is aerated (turned over) about 2 to 3 times per week. The heat generated by the fermentation results in moisture evaporation and drying. Moisture level is maintained at about 50-60% by adding more vinasse as needed. Although the vinasse is acid, the filter cake (pH 7.5-8.30) and the boiler ash bring the pH to the optimal range for the culture mix. It takes about 45 days to complete the process.

Incineration of Vinasse

On-Site Vinasse Disposal via Combustion

Concentrating vinasse from 10% solids to 60% solids produces a combustible material that can be used directly as fuel in the boilers. The process is energy intensive because of the need to evaporate a lot of water. To evaporate vinasse from 10% to 60% solids uses 0.16 ton of steam per ton of evaporated water (Cane Sugar Handbook, 12th Ed, 1993). For each ton of concentrated vinasse, 5.5 tons of water must be evaporated, or 0.9 tons of steam per ton of concentrated vinasse. This results in a net steam quantity available of 1.9 tons of steam per ton of burned concentrated vinasse. Therefore, burning the vinasse as fuel can be useful and profitable.

Heating values (kJ/kg):

Ethanol (96%)	27,837
Bagasse	10,410
Vinasse (60%)	7,600

It has been proposed to mix concentrated vinasse 1:1 with bagasse and drying the mixture with flue gas for burning. The ash from the furnace will be high in potassium and can be used as a fertilizer.

Vinasse alone will only combust above 70% solids, but when mixed with fuel oil, it will combust at 50% solids content (Cortez and Perez, 1997). A mixture of #6 fuel oil and vinasse was found to be optimal. Vinasse did not make a stable emulsion with diesel. The vinasse mixture was atomized in a hot furnace heated with natural gas and then fed into the combustion chamber. Best results were obtained with 75% to 95% fuel oil and 25% to 5% vinasse. Above 25% vinasse, the flame was not stable. Some

drawbacks of the operation were: (1) a lot of energy is required to evaporate the vinasse; (2) the vinasse can foam very badly during evaporation; (3) salts crystallize in the concentrated vinasse, causing pumping difficulties and evaporator scaling; (4) a lot of fused ash is formed, which has no use.

Anaerobic Digestion (AD)

Of all the avenues for vinasse disposal, anaerobic digestion (AD) holds the most promise. AD is the use of microbiological processes, in the absence of oxygen, to break down organic matter into methane, carbon dioxide and a stable solid residue. Ideally, the methane is recovered for its fuel value and the residue is returned to the field as a fertilizer or compost. Anaerobic treatment can be applied to most types of solid and liquid waste, including that from food processing, brewery, pulp and paper, slaughterhouse, dairy and wool processing. The use of anaerobic digestion for treating industrial wastewaters has grown tremendously during the past decade.

Anaerobic fermentation converts feedstock (plant material, stillage) into three products: Water, a nitrogen-rich compost, and digestor gas (biogas), which is composed of about 60% methane and 40% carbon dioxide. An AD system is most successful and stable when a variety of inputs (feedstocks) are used, such as molasses, velvet bean, bagasse, and cattle manure, which provides a balance of nutrients.

Many bacteria are involved in an anaerobic digestion system. The methane-producing bacteria are the Methanobacteria. Other types of bacteria are called homo-acetogenic, and include *Clostridium thermoaceticum*. Hydrolytic bacteria are also important because they break up the biomass, such as pectin, starch and cellulose.

Requirements for the anaerobic bacteria: (Tielbaard, 1992)
    Optimal temp is 33 - 38°C
    Optimal pH is 6.5 - 7.5
    Sufficient nutrients and micronutrients in the correct balance
    No toxic components in the mix

There are many possible reactor designs and choosing a good design is important. The most popular is the Upflow Anaerobic Sludge Blanket (UASB) process, which uses a smaller footprint than most other digestor designs and can handle larger volumes. The key to the success of the UASB digestor is the spontaneous formation of small granular bacterial pellets, or granules, in the reactor (Schmidt and Ahring, 1996).

As with all things that purport to be the answer, there are potential problems and considerations. Anaerobic digestion can be difficult to control and is highly subject to the composition of the vinasse, the pH, weather, etc. BOD is generally reduced 60-70%, even though there is a potential for up to 85% reduction. The composition of the feedstock, that is, having the right balance of carbon and nitrogen is critical, as is the pH and temperature. Control of potentially toxic constituents, such as phenolics, volatile fatty acids and heavy metals is important, as the organisms in the reactor are sensitive and may be killed.

It takes about 48 days to get a reactor operational. COD removal in an anaerobic digester can range from 65-93%.

In the future, as more and more vinasse is produced, and there is greater dependence on ethanol for energy, it may become feasible to consider some of the vinasse remediation steps discussed earlier in this paper. Or even to imagine that some of the remediation steps may be applied earlier in the process, that is, to molasses prior to fermentation.

In India, there were a lot of problems getting a UASB reactor to work properly (Tielbaard, 1992). Even at 1:1 dilution the vinasse was toxic to methanogenic bacteria because of the high potassium, sulfide and volatile fatty acids present in the vinasse. The sulfide resulted from the reduction of sulfate by the organisms. However, the same authors reported a successful start-up in Venezuela that was able to remove 65-70% of the COD

Work on a Colombian Vinasse

A detailed study was done by SPRI in cooperation with Sucromiles and Cenicaña on the composition of organic compounds in Colombian vinasse (Morales, et al, 2002). Table 4 shows that the vinasse was rich in several compounds. Many other compounds of interest were identified in smaller amounts (Table 5). These results show the potential for harvesting chemicals from vinasse in the future.

Table 4. The major organic compounds in a Colombian vinasse. (Ash = 12.4% of dry matter)

Compound	% of dry matter
Polysaccharides	5.21
Colorant polymers >12,000 Da	2.31
Glycerol	4.17
Sorbitol	2.15
Myoinositol	0.56
Trehalose	0.47
Sucrose	0.32
Fructose + Glucose	2.00
Aconitic acid	2.71
Citric acid	1.24
Lactic acid	1.97
Quinic acid	1.09
Malic acid	0.35
2,4-Dihydroxy-pentanedioic acid	1.09
Butanediol	0.32

Table 5. Other compounds identified in vinasse.

Acetic acid	Ethyl succinate	4-Methylcyclohexanol
Acetone	Formic acid	5-Methyl furfural
Alanine	Fumaric acid	2-Methylfuran
Aspartic acid	2-Furancarboxylic acid	Palmitic acid
Benzaldehyde	Furfuryl alcohol	2-Phenyl-ethanol
Benzoic acid	Glyceric acid	Phenylethyl alcohol
Butanoic acid, butyl ester	Glycolic acid	Phenyl lactic acid
2,3-Dihydrobenzofuran	1-Hydroxyacetone	Propylene glycol
2,3-dihydro-3,5-dihydoxy-6-methyl-4H-pyran-4-one	p-Hydroxy benzoic acid	Pyroglutamic acid
	3-Hydroxy-2-butanone	Pyrrolyl ethanone
2-5-Dimethylfuran	p-Hydroxy cinnamic acid	Resorcinol
2,4-Dimethyl-4-OH-3-(2H)-furanone	2-OH-furancarboxylic acid	Stearic acid
	2-Hydroxy hexanoic acid	Succinic acid
2,6-Dimethoxyphenol	Itaconic acid	Syringic acid
Dimethylsulfide	Methoxyphenyl ethanone	3,4,5-Trimethylpyrazole
Ethanol	2-Methylbutanal	Vanillic acid
Ethyl palmitate	3-Methylbutanal	Xylitol

Conclusion

Vinasse produced from cane juice or cane molasses alcohol fermentation is produced in volumes 10 to 15 times that of the alcohol, and is a disposal problem. Several innovative and potentially promising treatment methods have been developed and are undergoing further study and improvement. Vinasse is a complex product with potentially valuable residues of several organic constituents.

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Anaerobic Digestion of Vinasse for the Production Of Methane in the Sugar Cane Distillery

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Abstract

The expansion and diversification of new alternative energy sources in a sustainable and efficient way figures strongly among the major concerns of the industrialized world. Recent energy supply crunches and price spikes have propelled ethanol as an alternative transportation fuel. Ethanol derived from renewable sources has brought a host of challenges along with opportunities to the sugar industry. Providing cost effective systems for the treatment, conservation and recycling of water and energy resources is definitively one of these challenges.

Vinasse, the liquid residue left in the distillation of ethanol from sugar cane derivates, frequently poses serious disposal challenges as evidenced by its high biochemical oxygen demand (BOD). On an average basis, 12 cu ft of vinasse per cu ft of ethanol are produced in the distillery, with a BOD load ranging from 1.06 to 3.12 lb/cu ft of vinasse (17,000 to 50,000 mg/l). A study was carried out to assess the anaerobic digestion of vinasse for the production of methane. The purpose of this study was to gather enough data for subsequent evaluation of the technical feasibility of the process. The anaerobic digestion featured a complete mix reactor (digester) utilizing a two steps acid and methane-producing bacteria (thermophilic). Calculations data included temperature of 40°C (104°F) and mean cell residence time of 10 days. Results of a mathematical anaerobic digestion model (MADM) built to evaluate the system indicated that a 90% BOD reduction in the vinasse could be obtained by anaerobic digestion in a sugarcane-to-ethanol distillery producing 1,500,000 cu ft per year of ethanol (38,000,000 l per year).

As a byproduct of the digestion process, methane (CH_4) and other gases are produced in quantity enough to generate 3.6 to 10.60 megawatt of electricity (assuming 90% thermal efficiency), when vinasse BOD ranged from 1.06 to 3.12 lb/cu ft, respectively (17,000 to 50,000 mg/l). The gas production per weight of BOD destroyed was 8.92 cu ft/lb, while the food to microorganism (F/M) was 31.03 lb BOD/lb cell mass. In addition, daily production of cellular mass ranged from 3,658 to 10,758 lb/day (for a yearly operation of 150 days) when vinasse BOD ranged from 1.06 to 3.12 lb/cu ft (17,000 to 50,000 mg/l). The volume of the digester ranged from 1,048,836 to

440,857 cu ft (29,697 to 12,482 kl) when vinasse BOD ranged from 1.06 to 3.12 lb/cu ft (17,000 to 50,000 mg/l). Calculated data indicate that the productivity ratio of methane-vinasse ranged from 5.11 to 15.03 cu ft of methane per cu ft of vinasse digested and that of methane-ethanol ranged from 61.31 to 180.32 cu ft of methane per cu ft of ethanol.

Introduction

At the turn of the 20th Century, energy supply crunches and price spikes focused attention on the need for industrial process improvement and development of alternative energy sources such as ethanol fuel (Renewable Fuel Association, 2004). Recent breakthroughs in enzyme technology and processing are radically changing the viability of ethanol as a transportation fuel. However, pressing economical constraints and environmental regulations have placed a demand for increased productivity and diversification of the industrial plant byproducts portfolio. Segregating the less valuable fractions for use as fuel, thus creating value-added products, appeals to present productivity demands. Sugar cane distillery waste disposal improvements are strongly needed, as evidenced by vinasse, or stillage, which is the liquid residue left after distillation of alcohol.

The average production of vinasse in the sugarcane distillery is approximately 12 gallons of vinasse per gallon of ethanol, which represents an enormous volume of wastewater for disposal. When evaluated in population terms or a per capita basis, a distillery with a daily production of 110,000 gallons of ethanol is equivalent to wastewater production for a city with a population of approximately 768,000 people. However, it is not necessarily the volume of vinasse but restrictions for effluent's biochemical oxygen demand (BOD) discharge by current environmental laws and regulations that seems to present the biggest challenge to the profitable use and disposal of vinasse.

Among available technology, anaerobic digestion is one of the dominant BOD reducing processes in wastewater treatment, since gas of high calorific value is produced, as well as relatively inoffensive sludge suitable for use as a fertilizer. A mathematical anaerobic digestion model (MADM) was developed to evaluate the anaerobic digestion of the vinasse as a way of improving the sugar-to-ethanol distillery productivity.

The purpose of the MADM was to gather enough data for subsequent evaluation of the technical feasibility of vinasse anaerobic digestion. Data gathering included key design parameters for the process of anaerobic digestion, such as digester or reactor volume, mass of cellular tissue, total volume of gas produced, as well as the electrical power capabilities of the gas produced.

Since the design of anaerobic digestion processes is based on fundamental principles of microbiology, biochemistry and kinetics engineering, the scope of this study will present (1) a background overview of microbiological and biochemical process fundamentals governing biological growth and waste treatment kinetics (2) a brief description of vinasse itself, (3) a description of the methodology used in the development of the MADM, (4) results of the mathematical model (MADM) output (5) discussions of results and (6) conclusions.

Background

Microbiological and Biochemical Overview

Anaerobic treatment can be defined biochemically as the conversion of organic compounds into carbon dioxide, methane and microbial cells (sludge), in the absence of free or molecular oxygen (Corbitt, 1989). For anaerobic treatment of organic nitrogen compounds, the end products will also include ammonia (Appendix A, equation A- 2).

The oxidation and reduction reactions occurring in the anaerobic breakdown of organic matter are as follows (Klein et al, 1972):

$$Carbon, C \rightarrow organic\ acids\left(R \bullet COOH\right) \rightarrow CH_4 + CO_2$$ **Equation 1**

$$Nitrogen, N \rightarrow amino\ acids\left[R \bullet \left(NH_2\right) \bullet COOH\right] \rightarrow NH_3 + amines$$ **Equation 2**

$$Sulphur, S \rightarrow H_2S + organic\ S\ compounds$$ **Equation 3**

$$Phosphorus, P \rightarrow PH_3 + organic\ P\ compounds$$ **Equation 4**
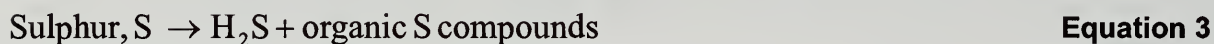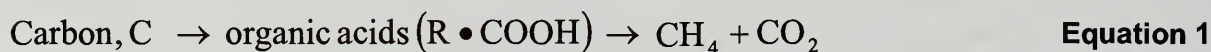
Vinasse Characteristics

Composition Profile

Vinasse represents a mixture of water, organic and inorganic compounds (Cortez and Brossard Pérez, 1997). The mixture is dependent on the raw material used in the alcohol fermentation process. The temperature of the vinasse is in the range of 65° to 105° C (149-221°F). Vinasse has a light brown color with a solid content from 20,000 to 40,000 mg/l when obtained from straight sugarcane juice, and a black-reddish color with total solids ranging from 50,000 to 100,000 mg/l when obtained from sugarcane molasses. In addition, vinasse is an acidic liquid with pH between 4 and 5 and high chemical oxygen demand (COD) content (Tables 1 and 2). The inorganic solids contain considerable amounts of nutrients such as phosphorus, nitrogen and potassium (Table 1).

Environmental Limits

Many researchers (Cortez and Brossard Pérez, 1997; Barreto de Menezes, 1980; Paturau, 1969) have reported major environmental problems for the appropriate disposal of vinasse, which is understandable given the fact that BOD effluent discharge limits for most of environmental regulations throughout the world range from 30 to 100 mg/l for water and land disposal methods (Corbitt, 1989). Furthermore, the hydrogen sulfide, amines and other offensive-smelling chemicals that are generated by decomposition of the organic matter seem to add to vinasse's reputation as a difficult residual effluent. In Brazil, vinasse is disposed as irrigation water (Donzelli and de Souza, 2003). However, in other countries, vinasse treatment before disposal is required by federal and state environmental regulations due to its high BOD concentration (Appendix B, Tables B-1, B-2 and B-3).

Table 1. Comparative composition of vinasse derived from sugar cane

	Brazil (1)	Brazil (2)	Australia (1)	Australia (2)	India	USA (La)
Component	Juice	Molasses	Molasses	Molasses	Molasses	Molasses
K, mg/l	1,733	4,893	8,767	10,704	4,078	9,073
P, mg/l	71	102	20	12	5,097	1
N, mg/l	102	408	3,160	1,835	1,019	153
Ca, mg/l	408	714	1,121	2,039	n.a.	143
Mg, mg/l	102	204	1,529	1,325	n.a.	61
Ash, mg/l	15,292	19,879	32,622	n.a.	n.a.	50,972
Organic Solids, mg/l	52,399	47,200	n.a.	n.a.	n.a.	n.a.
Total Solids, mg/l	68,201	n.a.	n.a.	91,750	69,322	n.a.
pH	4.6	4.8	n.a.	n.a.	4.3	4.5

Source: Cortez, L.A.B., and L.E. Brossard Perez. Experiences on vinasse disposal. Part III: Combustion of vinasse-#6 fuel oil emulsions, Brazilian Journal of Chemical Engineering, Vol 14, No.1, 1997, São Paulo, Brazil.
Note: Data converted to mg/l from original % composition
n.a. means not available

Table 2. Vinasse composition used for modeling study

Component		
	Range	Range
pH	4-5	4-5
	mg/l	lb/cu ft
BOD	17,000 - 50,000	1.06 - 3.12
COD	20,000 - 60,000	1.25 - 3.75
Total solids	30,000 -70,000	1.87 - 4.37
Total nitrogen	300-800	0.01 - 0.05
Total phosphorus (as phosphates)	100-500	0.01 - 0.03
Total potassium (K_2O)	2,000 - 3,000	0.12 - 0.19
Ash	3,000 - 10,000	0.19 - 0.62

Adapted from Cortez, L.A.B., L.E. Brossard Perez, Experiences on Vinasse Disposal, Part III: Combustion of vinasse-#6 oil emulsions, Brazilian Journal of Chemical Engineering, Vol. 14, No. 1, 1997, São Paulo, Brazil.

Methodology

Anaerobic Digestion of Vinasse

Process Description
Operationally, biological waste treatment is typically accomplished using a digester such as that shown in Figure 1, which is proposed for the digestion of vinasse. The process apparatus consist of several basic components, including a feedstock (vinasse) storage and handling system, digester tank (reactor), gas and residue recovery systems, and if electricity is to be produced, a gas-burning engine/generator set.
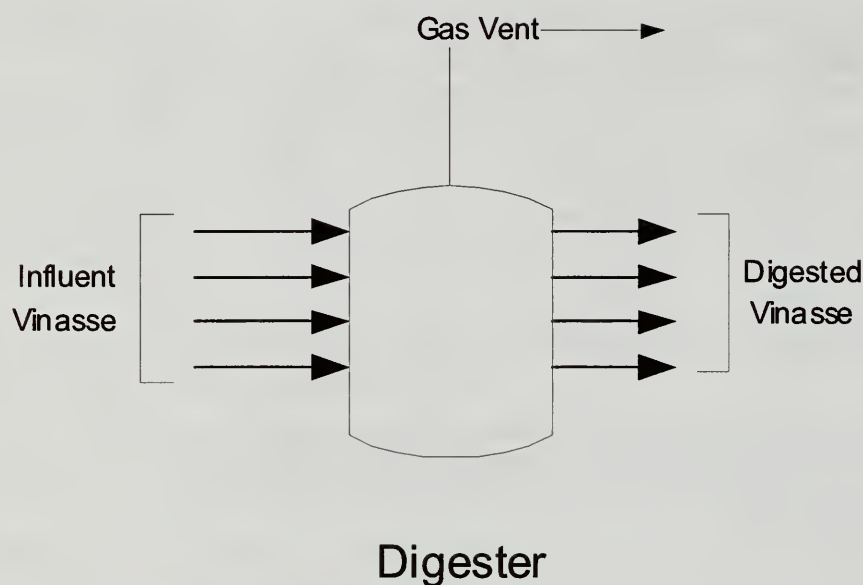
Digester

Figure 1. Schematic representation of digester used for anaerobic digestion.

The process starts as vinasse is introduced into the reactor where anaerobic bacterial culture is maintained in suspension. Temperature of influent vinasse was assumed as 40° C (104° F). The process is carried out in an airtight complete-mix reactor. Vinasse is retained in the reactor for an average period of time equivalent to 10 days, which is the literature-recommended residence time for anaerobic digestion at 40°C in a complete mix reactor (Metcalf and Eddy, 1991). The bacterial culture carried out the conversion of the organic material to a variety of end products including methane (CH_4), carbon dioxide (CO_2), ammonia (NH_3) and cell or bacterial mass. As the methane gas is highly insoluble in water, the resulting gas mixture is collected in the dome of the reactor and withdrawn for energy recovery. The resulting cell tissue can be removed from the treated liquid by gravity settling because cell tissue has a specific gravity slightly greater than that of water (Metcalf and Eddy, Inc., 1991). The effluent or digested vinasse withdrawn from the reactor is reduced in organic load (BOD) by 90%. Digested vinasse is basically a mix of water and inorganic and organic compounds.

The bacteria chosen for the process were both, mesophilic and thermophilic. For the purpose of this study, (Table 2) BOD concentration in vinasse was assumed to be in the range of 1.06 to 3.12 lb/ft^3 (17,000 to 50,000 mg/l). The anaerobic digestion of organic matter is an oxidation-reduction reaction involving water and occurring within the pH range 6.5-8.0.

Mathematical Model (MADM)

A mathematical model using Microsoft Excel® (MADM) was developed with the purpose of evaluating the design parameters for this study. The MADM was configured in such a way that an output is provided for any size of ethanol producing facility input in the model data or for any change in the data fed to the model. In addition, the MADM has the capabilities of calculating the tonnage of cane required for the production of the ethanol, based on sucrose content in sugar cane and fabrication extraction parameters.

Calculations featured anaerobic digestion of vinasse for a distillery producing 10 million gallons/year of ethanol (37,850,000 l/yr), for which the calculated amount of vinasse was approximately 120,000,000 gallons/year. Vinasse production was equivalent to 800,000 gallons of vinasse per day (3,028,000 l/day) for 150 days of operation, 7 days per week and 24 hours per day.

Several equations were input into the mathematical model for the calculation of the anaerobic digestion process parameters, which are the following:

Volume of Methane

Equation 5 calculated the volume of methane

$$V_{CH_4} = (5.62)[(S_o - S)(Q)(8.34) - 1.42 P_x] \qquad \textbf{Equation 5}$$

Where V_{CH4} = volume of methane produced at standard conditions (32° F and 1 atm), cu ft/day
5.62 = theoretical conversion factor for the amount of methane produced from the complete conversion of one pound of BOD to methane and carbon dioxide, cu ft CH_4/lb BOD oxidized
Q = flow rate, Mgal/day
S_o = ultimate BOD in influent, mg/l
S = ultimate BOD in effluent, mg/l

Mass of Cellular Tissue Production during Digestion

For a complete-mix, high rate digester without recycle, the mass of biological solids synthesized daily (P_x) was calculated using Equation 6 (Metcalf & Eddy, Inc., 1991).

$$P_x = \frac{Y[(S_o - S)(Q)(8.34)]}{1 + k_d \theta_c} \qquad \textbf{Equation 6}$$

Where   Y = yield coefficient, lb/lb
K_d = endogenous coefficient, day^{-1}
θ_c = mean cell-resident time, day
Other terms are as defined above.

The kinetic coefficient (endogenous coefficient) recommended in the literature for substrate similar in composition to vinasse (fatty acid) was 0.04 d^{-1} and the yield coefficient was 0.05 lb of cell/lb of BOD (Metcalf & Eddy, Inc. 1991).

Digester Volume

The volume of the digester was calculated (Appendix C) using Equation 7 (Levenspiel, 1972; Metcalf & Eddy, Inc., 1991).

$$V_r = Q(\theta_c)$$ **Equation 7**

Electrical Power

The volume of methane generated during the anaerobic digestion was used to calculate the electrical energy output. Methane gas at standard temperature and pressure has a net heating value of 960 Btu/cu ft. The low heating value of digester gas is roughly 600 Btu/cu ft, because digester gas is a mixture of approximately 60% methane and the rest being CO_2, hydrogen sulfide, particulates and water vapor (Metcalf and Eddy, 1991). By comparison, natural gas, which is a mixture of methane, propane and butane, has a low heating value of approximately 1000 Btu/cu ft.

The net electrical output was calculated using Equation 8 (Kirby, 2003).

$$\eta_{Overall} = \frac{\text{Net Electric Output}}{\text{Fuel Input}}$$ **Equation 8**

The overall thermal efficiency used for calculation purposes was 90%.

Performance Parameters

The MADM also evaluated several parameters of fundamental importance for anaerobic digestion. Among these parameters are the organic loading rate (volumetric loading rate), the volume of gas produced per pound of BOD in the influent and the food to microorganism (F/M) ratio.

The volumetric loading rate is expressed as the weight of organic or volatile feed sludge added per volume of digester per day, for example, lb BOD/cu ft day.

The volume of gas produced per pound of BOD in the influent is expressed as cu ft of gas/lb BOD destroyed in the vinasse influent, while the F/M ratio was expressed as lb BOD in the vinasse influent/lb of cellular tissue produced.

In addition, two gas productivity parameters were evaluated from the model's results. One of these parameters was the volume of methane gas produced per volume of ethanol produced, which was expressed as cu ft methane/cu ft of ethanol. The other parameter was the volume of methane gas produced per volume of vinasse processed, which was expressed as cu ft methane/cu ft of vinasse.

Basic Assumptions

1. It was assumed that the vinasse BOD concentration was reduced by 90% during anaerobic digestion. Due to vinasse strategic composition of organic acids, the digester efficiency of waste utilization should be much higher (90 to 97% of BOD content) than that of conventional organic sludge (60 to 70% of BOD content), since steps (1) and (2) of the three stage oxidation-reduction

anaerobic digestion are already completed before entering the reactor (Appendix A, Figure A-1). Several researchers (Marshall and Kopp, 2006 and Manohar Rao, 1999) have reported BOD reduction of 90% or more for the anaerobic digestion of vinasse.

2. It was assumed that anaerobic digestion of vinasse takes as little as 10 days, instead of the 30 to 40 days taken by conventional anaerobic digestion. Since vinasse is free of heavy metals and other toxic materials found in conventional wastewater sludge, better digestion process leading to shorter residence time can be expected.

3. It was assumed that the concentration of microorganisms in the influent was negligible.

Calculation Overview

The digester data and Equations 5 through 8 were input into the MADM and calculations were performed for constant volumes of vinasse in the digester feed while varying concentration of BOD in the vinasse. Influent vinasse BOD concentrations ranging from 1.06 to 3.12 lb/cu ft (17,000 to 50,000 mg/l) were used for the calculations (Table 2). In addition, vinasse was assumed to contain 1.87 to 4.37 lb/cu ft (30,000 to 70,000 mg/l) of total solids and moisture content ranging from 93 to 97%. Vinasse specific gravity was assumed to be in the range of 1.02 to 1.04.

Results and Discussion

Mathematical Model Output

The MADM output provided the calculated parameters for the anaerobic digestion of vinasse. The model results for the featured distillery are shown in Table 3 and Figures 2 through 4.

Calculated Parameters Data

Calculated data (Table 3) indicate that when vinasse BOD concentration ranged from 1.06 to 3.12 lb/cu ft (17,000 to 50,000 mg/l) the digester volume for vinasse anaerobic digestion decreased from 1,048,836 to 440,857 cu ft (29,697 to 12,482 kl). In addition, the digester volume decreased exponentially with BOD concentration increase (Figure 2), which is expected for the graphical representation of the design equation for a mixed reactor (Levenspiel, 1972).

Calculated data (Table 3) indicate that a high-rate reactor is required for anaerobic digestion of vinasse, since volumetric loading rates ranged from 0.11 to 0.76 lb/cu ft day (1.73 to 12.13 mg/l day). Literature data (The water Pollution Control Federation, 1976; Metcalf & Eddy, Inc., 1991) designate high-rate digesters as those having loading rates ranging from 0.10 to 0.40 lb/ft^3 day when the hydraulic detention period ranged from 10 to 20 days, which is in agreement with the digester used for this study (hydraulic retention time of 10 days).

Table 3. Results of the MADM calculated parameters for anaerobic digestion of vinasse

	Influent vinasse BOD, mg/l / lb/cu ft			
	17,000	**30,000**	**40,000**	**50,000**
Calculated Parameter	**1.06**	**1.87**	**2.50**	**3.12**
Influent vinasse BOD volumetric loading, lb/cu ft day	0.11	0.32	0.56	0.76
Influent vinasse BOD volumetric loading, mg/l day	1.73	5.15	8.97	12.13
Digester volume, cu ft	1,048,836	623,192	477,063	440,857
Digester volume, kl	29,697	17,645	13,507	12,482
Net mass of cell tissue (Px), lb/day	3,658	6,455	8,606	10,758
Volume of methane produced, cu ft/day	546,387	964,213	1,285,617	1,607,021
Volume of methane produced, l/day	15,470,283	27,300,499	36,400,665	45,500,831
Volume of gas produced, cu ft/day	910,645	1,607,021	2,142,695	2,678,369
Volume of gas produced, l/day	25,783,804	45,500,831	60,667,775	75,834,718
Effluent vinasse BOD , mg/l	1700	3000	4000	5000
Effluent vinasse BOD , lb/cu ft	0.11	0.19	0.25	0.31
Power generated, megawatts	3.60	6.36	8.48	10.60
Gas production/weight of volatile solids destroyed, cu ft/lb	8.92	8.92	8.92	8.92
Gas production/weight of volatile solids destroyed, l/mg	0.56	0.56	0.56	0.56
Food to microorganisms ratio, mg BOD/mg cell mass	31.03	31.03	31.03	31.03
Productivity rate, cu ft methane/cu ft ethanol, l/l	61.31	108.19	144.26	180.32
Productivity rate, cu ft methane/cu ft vinasse, l/l	5.11	9.02	12.02	15.03
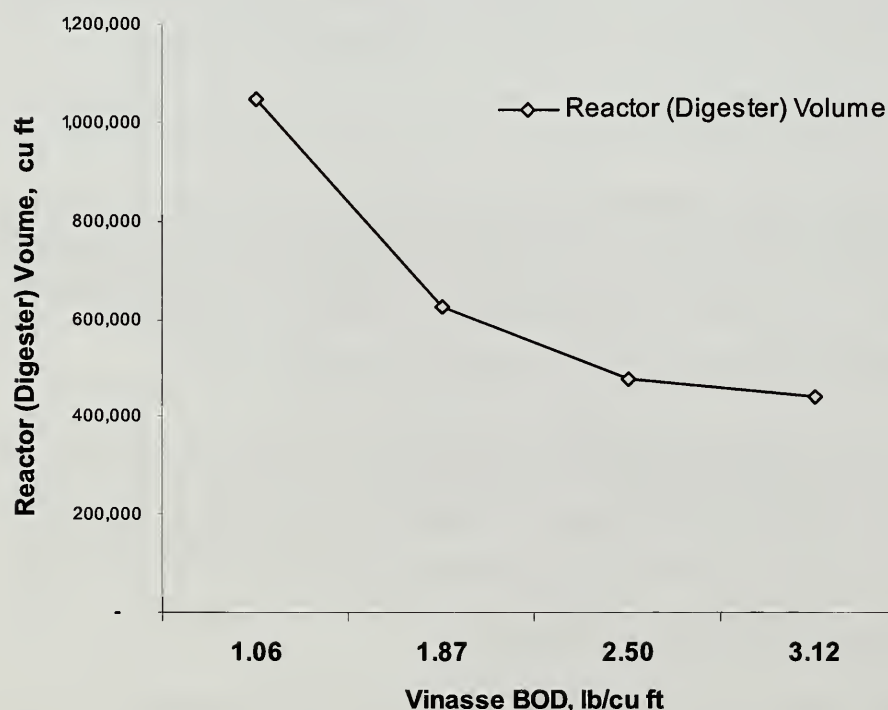
Figure 2. Reactor (digester) volume for anaerobic digestion

The calculated gas production per lb BOD destroyed was 8.92 cu ft/lb (Table 3), which is in agreement with values of 8.0 to 18.0 cu ft /lb found in the literature for typical anaerobic digestion of organic matter (The Water Pollution Control Federation, 1976, Metcalf & Eddy, Inc., 1991).

In addition, the calculated F/M ratio was 31.03 lb BOD/lb cell mass (Table 3). A high F/M ratio (20 to 30 lb BOD/lb cell mass) is beneficial for rapid removal of the soluble organic in the influent, since it provides high substrate driving force for quick absorption into the cellular mass (Metcalf & Eddy, Inc., 1991).

The volume of methane and total gas produced was nearly proportional to the rate of organic loading (Figure 3). The Water Pollution Control Federation (1976) reported similar results, which stated that the former is true for both the average 24-hour loading rate and the instantaneous loading rate.

The power generation capabilities as a result of anaerobic digestion ranged from 3.60 to 10.60 megawatts (Figure 4), which seem to indicate that the power generated could satisfy the power requirements of the entire distillery. This represents significant savings for the production of ethanol from sugarcane. The power output is also proportional to the organic loading rate, as expected (Figure 4).
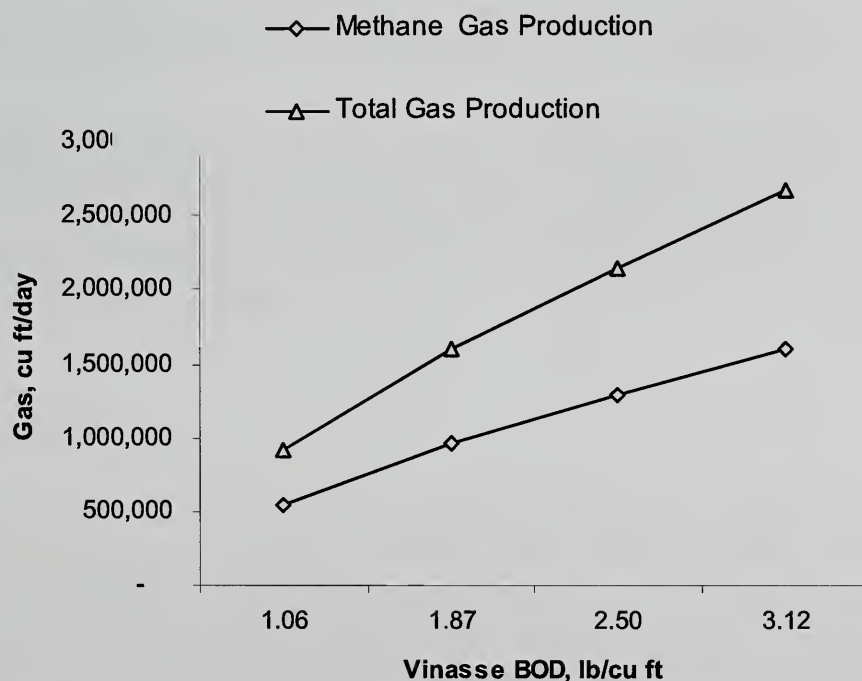
Figure 3. Gas production from anaerobic digestion of vinasse

There are several options for the gas produced, which provide great payback to the sugarcane distillery. If a gas turbine is installed, the methane can be used to produce "green" power for the distillery. In addition, the turbine exhaust gases can generate steam needed for the ethanol distillery. The biogas that is not converted into electricity can be scrubbed to pipeline specifications natural gas, compressed and injected into the existing natural gas distribution grid. Thus, the distribution lines are used as the storage system (Ethanol Producer Magazine, 2006).

The volume of methane gas produced per volume of ethanol produced ranged from 61.31 to 180.32 cu ft methane/cu ft ethanol when BOD in vinasse ranged from 1.06 to 3.12 lb BOD/cu ft vinasse (17,000 to 50,000 mg/l). The volume of methane gas produced per volume of vinasse processed ranged from 5.11 to 15.03 when BOD in vinasse ranged from 1.06 to 3.12 lb BOD/cu ft vinasse (17,000 to 50,000 mg/l).

Calculations by the MADM represent methane generated from BOD conversion only, however, it have been reported that 70 % or more of the COD is also converted to methane (Manohar Rao, 1999), therefore, the methane capabilities of the process are expected to be greater than the one shown (Figure 3), which further complements the process productivity. However, pretreatment by ozone before anaerobic digestion might be needed to destroy phenolic compounds present in vinasse, which could be toxic to the anaerobic bacteria (Santos M. et al, 2003).
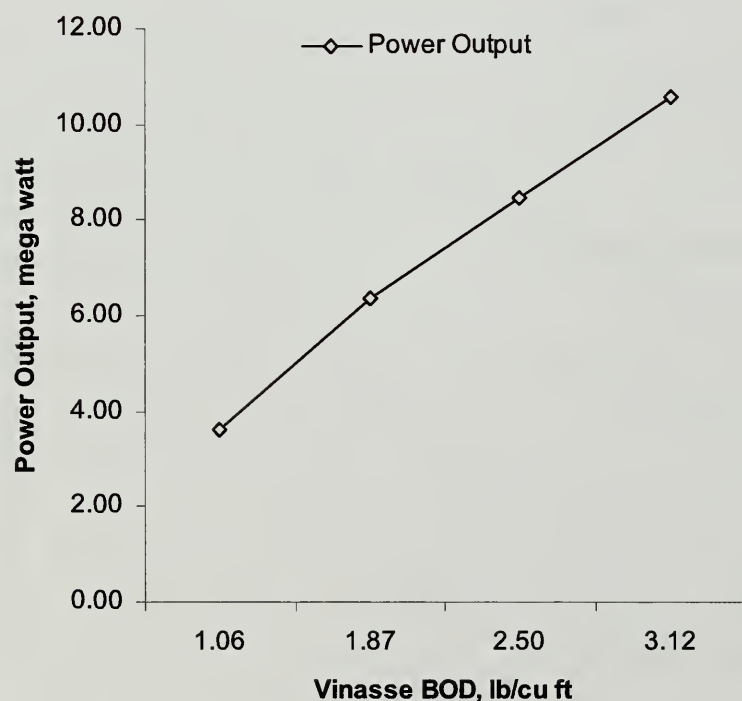
Figure 4. Power output using gas from anaerobic digestion

As the BOD content of the digester effluent vinasse is ranging from 0.11 to 0.31 lb/cu ft (1,700 to 5,000 mg/l), it is still over the range for allowed BOD effluent discharge for most environmental regulations throughout the world (30 to 100 mg/l). The residual effluent vinasse must be further processed to reduce the BOD and COD content for appropriate disposal (Table 3). However, this hurdle presents further opportunities for energy recovery. Concentration of the digested vinasse, either by evaporation or ultrafiltration could produce additional energy for the distillery. If the digested vinasse is evaporated to 65% solids, energy could be recuperated by burning the digested vinasse. If the vinasse is ultra-filtrated, potable water could be produced from the filtrate stream, while the concentrated stream could be burned for energy recovery or sold as a liquid fertilizer. The ash left after burning could be used as a fertilizer as well. Due to its high mineral content, vinasse creates optimum environmental conditions for the microorganisms involved in the digestion process, thus resulting in savings on process nutrient requirements and process economics.

Since the generation of biogas constitutes a non-conventional energy source, extra benefits could be obtained through the utilization of incentives and funding currently available from government legislation aimed at facilitating non-petroleum derived energy production. In addition to the clean ecological benefit, this process promotes energy savings and recovery.

Furthermore, extra energy savings in the distillery could be obtained by using vinasse to heat process water. Vinasse that comes out of the distillation process is in the range of 65° to 105° C (149° to 221° F) and it needs to be cooled to about 40° C (104° F). Therefore a heat exchanger can be used to lower the temperature of the hot vinasse with cool process water. At the same time, the vinasse warms the process water for use in the distillery.

As the sugar distillery process constitute an intense user of surface water, the production of potable water from the digested vinasse sludge could further decrease production costs in the distillery, in addition to the potential of achieving zero waste generation and discharge. The creation of all these value-added products will certainly boost the reduction in production cost of the sugar-to-ethanol distillery.

Conclusions

The gas as well as the electrical energy produced by anaerobic digestion of vinasse is proportional to the concentration of BOD in the vinasse influent. The volume of the digester decrease exponentially when BOD concentration in the influent vinasse increases. Therefore, optimal reactor size is obtained when BOD concentration in the influent vinasse is the highest. The implementation of the anaerobic digestion of vinasse will improve distillery productivity, as green energy is produced at the same time that other byproducts could also be produced.

The (MADM) is an effective tool for predicting the process of anaerobic digestion of vinasse, as calculated parameters correlated with available experimental data. Bio-methane production from the sugar distillery is an excellent process improvement project, as it has the potential to meet the fuel and steam requirements of the distillery, in addition to the potential environmental benefits. Greenhouse gas emissions could be reduced from replacing existing fossil fuel sources. In

addition, the production of "green" electrical power could earn further economic benefits by qualifying the distillery for available government-sponsored credits and other incentives.

Anaerobic digestion of vinasse constitute a good strategy for improving the disposal conditions of vinasse, since it reduces the BOD content, while gas of high calorific value is produced. With the ever-escalating energy prices, the methane produced could represent reduction of production costs of the distillery.

The implementation of such an intensive energy recovery, water recycling and value-added byproducts process such as the one encompassed by anaerobic digestion of vinasse, is certainly bound to create a huge improvement in the public relations image of the sugar industry. In addition to obtaining economical credits by the creation of value-added product and green energy production, anaerobic digestion could certainly tip the balance positively into an economic feasible sugar-to-ethanol distillery.

Recommendations

Due to the relevance of vinasse on the sugar cane distillery, the following alternatives are worth pursuing:

- Evaluation of the economics and viability of the anaerobic digestion of vinasse by conducting a feasibility study, as the process provides an alternative to high-priced natural gas.

- Evaluation of the production of potable water and fertilizer from the digested vinasse, which can potentially create greater value-added products for the ethanol distillery. Mineral-rich vinasse is an excellent substrate for fertilizer production.

- Evaluation of the use of the cell tissue mass discarded from the anaerobic digestion process for use as an additive to fertilizer.

Acknowledgements

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APPENDIX A

Process Biochemistry

The various chemical reactions brought about by bacteria are due to the activity of enzymes or "ferments" elaborated by the bacterial cells. Test of different bacteria indicate that they are about 80% water and 20% dry material, of which 90% is organic and 10% inorganic. An approximate formula for the organic fraction is $C_5H_7O_2N$ (Metcalf and Eddy, Inc., 1991).

To continue to reproduce and function properly, an organism must have (1) a source of energy, (2) carbon for the synthesis of new cellular material, and (3) inorganic elements (nutrients) such as nitrogen, phosphorus, sulfur, potassium, calcium and magnesium. Organic nutrients (growth factors) may also be required for cell synthesis. Two of the most common sources of cell carbon for microorganisms are organic matter and carbon dioxide. The energy needed for cell synthesis may be supplied by light or by a chemical oxidation reaction.

Required organic nutrients, known as "growth factors," are compound needed by an organism as precursors or constituents of organic cell material that cannot by synthesized from other carbon sources. Among the major growth factors are amino acids, purines and pyrimidines, and vitamins (Metcalf and Eddy, Inc., 1991).

The anaerobic decomposition of organic matter is a three-stage reaction: (1) hydrolysis of the organic material into soluble organic compounds, (2) acetogenesis, or conversion of soluble organics to volatile fatty acids (mostly acetic acid); and (3) methanogenesis, or conversion of the volatile fatty acids into methane (Klein et al, 1972, Metcalf & Eddy, Inc., 1991).

The types of microorganisms involved in acetogenesis are often identified as "acidogens" or "acid formers" and among these are clostridium spp., peptococcus anaerobus, Bifidobacterium spp., Desulphovibrio spp., Corynebacterium spp., Lactobasillus, Actinomyces, Staphylococcus, and Escherichia coli. Other physiological groups include those producing proteolytic, lipolytic, ureolytic or cellulytic enzymes.

The bacteria responsible for methanogenesis are identified as "methanogens," or "methane formers (Metcalf & Eddy, Inc., 1991)." The principal genera of microorganisms that have been identified include the rods (Methnobacterium, Methanobacillus) and spheres (Methanococcus, Methanosarcina).
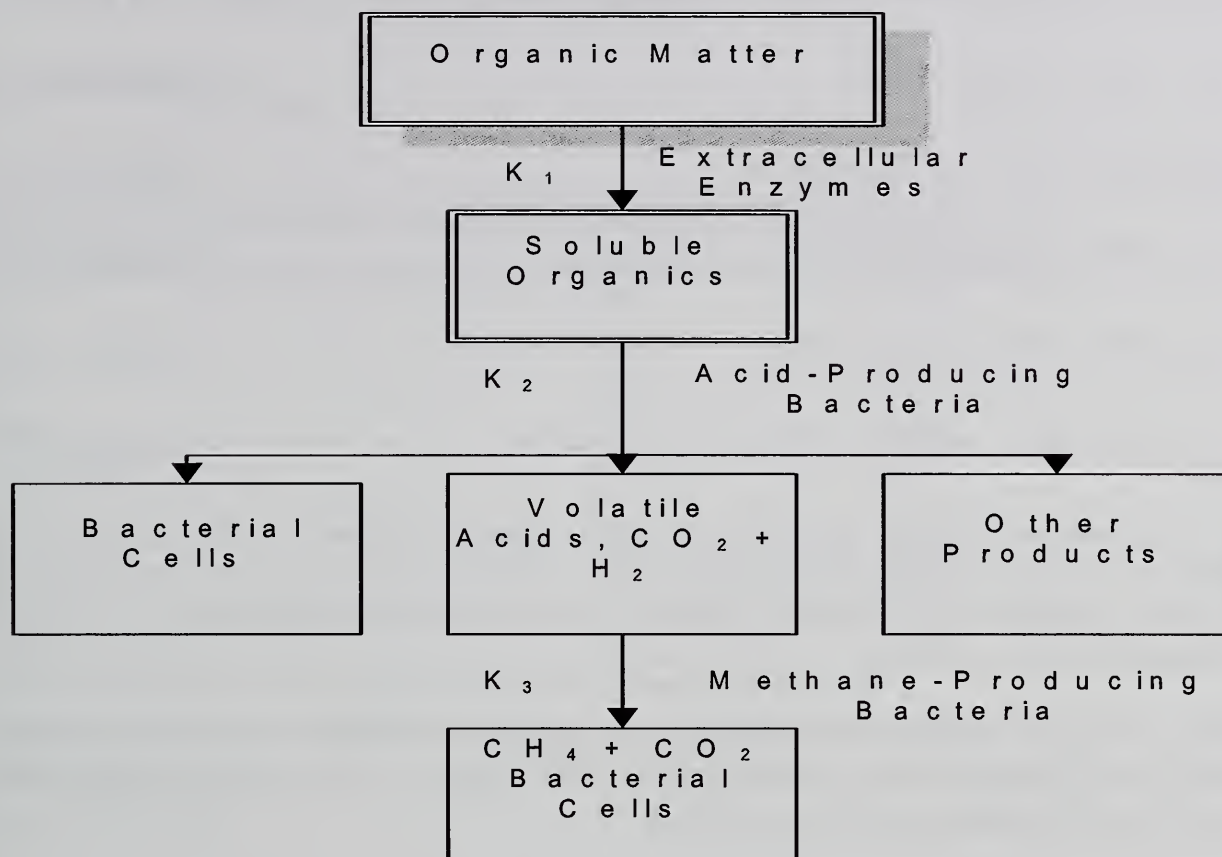
Anaerobic digestion of organic wastes. K_1, K_2 and K_3 refer to the rates of reaction.

Figure A-1. Stages of anaerobic digestion

In the digester the bacterial culture carries out the conversion in general accordance with elemental stoichiometric (Equation A-1).

$$C_nH_aO_b + \left(n - \frac{a}{b} - \frac{b}{2}\right)H_2O \rightarrow \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right)CO_2 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right)CH_4 \qquad \text{Equation A-1}$$
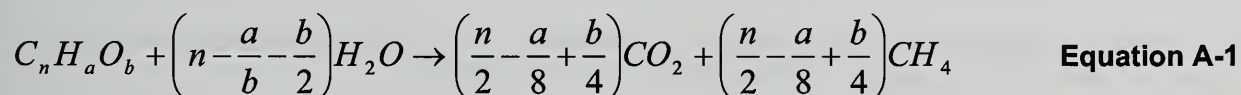
In the case of anaerobic digestion of acetic acid, Equation A-2 can represent the reaction:

$$C_2H_4O_2 + \left(2 - \frac{4}{4} - \frac{2}{2}\right)H_2O \rightarrow \left(\frac{2}{2} - \frac{4}{8} + \frac{2}{4}\right)CO_2 + \left(\frac{2}{2} + \frac{4}{8} - \frac{2}{4}\right)CH_4 \qquad \text{Equation A-2}$$
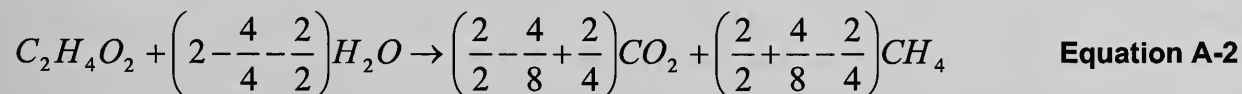
It is important to note that methane bacteria can only use a limited number of substrates for the formation of methane. Currently, it is known that methanogens use the following substrates: $CO_2 + H_2$, formate, acetate, methanol, methylamines, and carbon dioxide.

Typical energy-yielding conversion reactions involving these compounds are as follows:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \qquad\qquad \textbf{Equation A-3}$$

$$4HCOOH \rightarrow CH_4 + 3CO_2 + 2H_2O \qquad\qquad \textbf{Equation A-4}$$

$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad\qquad \textbf{Equation A-5}$$

$$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O \qquad\qquad \textbf{Equation A-6}$$

$$4(CH_3)_3N + H_2O \rightarrow 9CH_4 + 3CO_2 + 6H_2O + 4NH_3 \qquad\qquad \textbf{Equation A-7}$$
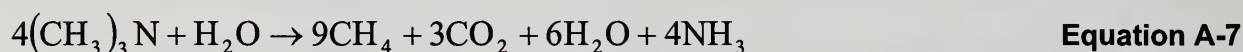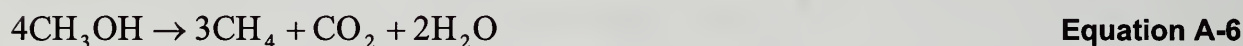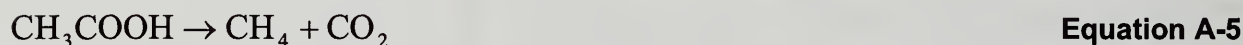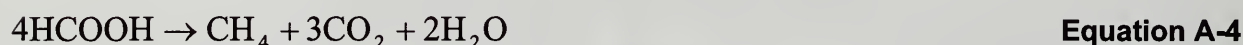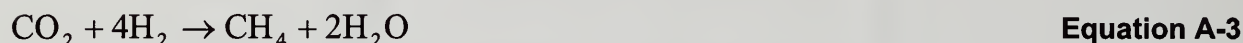
In an anaerobic digester, the two principal pathways involved in the formation of methane are (1) the conversion of hydrogen and carbon dioxide to methane and water and (2) the conversion of acetic acid to methane and carbon dioxide (Equations A-3 and A-5, respectively).

Most biological treatment processes are comprised of complex, interrelated, mixed biological populations, with each particular microorganism in the system having its own growth rate (Metcalf and Eddy, Inc., 1991).

Nutrient Requirements

If a biological system is to function properly, nutrients must be available in adequate amounts. The principal nutrients are nitrogen and phosphorus. Based on an average composition of cell tissue of $C_5H_7NO_2$, about 12.4 % by weight of nitrogen will be required. The phosphorus requirement is assumed to be about one-fifth of this value. However, these are just typical values, as the percentage distribution of nitrogen and phosphorus in cell tissue varies with the age of the cell and environmental conditions.

Other nutrients required, but only in trace quantities, are sodium, potassium, calcium, chloride, sulfate and bicarbonate (Metcalf & Eddy, Inc., 1991).

Vinasse typically contains adequate amounts of nutrients (both inorganic and organic) to support biological treatment for the removal of carbonaceous BOD. In Brazil, most of the vinasse that results from ethanol production is being used as fertilizer due to high potassium content (Donzelli, Penatti and de Souza, 2003).

APPENDIX B

Vinasse Composition

Table B-1. Dry basis vinasse composition (molasses derived vinasse)

Component	As Received	Dry Basis
	%	%
Solids	29.79	n.a
Ash	13.31	18.95
Sulphur	0.08	0.12
Volatile matter	48.67	69.31
Fixed carbon	8.24	11.73
Carbon	n.a	39.72
Hydrogen	n.a	8.6
Nitrogen	n.a	1.65

Source: Cortez, L.A.B., L.E. Brossard Perez, Experiences on Vinasse disposal, Part III:

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1997, São Paulo, Brazil.

n.a. means not available

Table B-2. Analysis of vinasse from various raw materials used for ethanol fermentation

Components	Raw Material		
	Molasses	Cassava	Sorghum
pH	4.4	3.5	4.5
	mg/l	mg/l	mg/l
BOD	25,800	31,400	46,000
COD	48,000	81,100	79,900
Total Solids	68,000	44,500	34,100
Soluble Solids	57,100	40,400	n.a.
Fixed Solids	48,400	4,100	n.a.
Suspended solids	38,700	n.a.	n.a.
Organic matter	19,500	37,100	n.a.
Carbohydrates	8,000	20,100	3,400
Total Nitrogen	820	650	800
Total phosphorus (as phosphates)	480	380	100
Ash	10,700	10,500	6,100

Source: Barreto de Menezes, T. J., Etanol, o Combustible do Brasil (Ethanol, Brazil's fuel, in Portuguese language),

1980, Editora Agronomica Ceres, Ltda, São Paulo, Brazil.

n.a. means not available

Table B-3. Dry basis vinasse composition (sugar cane juice vinasse)

Compound Name	Compound Amount
	%
Mineral matter	29
Sugar (reducing)	11
Proteins	9
Volatile acids	1.5
Gums	21
Combined lactic acid	4.5
Other combined organic acids	1.5
Glycerol	5.5
Wax, phenolic bodies, lignin, etc.	17

Source: Paturau, J.M., By-Products of the Cane Sugar Industry, an Introduction to their Industrial Utilization, 1969, Page 183, Elsevier Publishing Company, New York.

APPENDIX C

Process Design

Various methods are currently used for digester design and these are based on (1) the concept of mean residence time, (2) the use of volumetric loading factors, (3) observed volume reduction, and (4) loading factors based on population. For the purpose of this investigation, the concept of mean residence time will be considered. In addition, the anaerobic treatment process will be carried out in a complete-mix reactor without recycle.

A mass balance for the mass of microorganisms in the complete-mix reactor can be written as follows:

$$\begin{pmatrix} Rate\ of\ accumulation \\ of\ microorganisms \\ within\ the\ system \\ boundary \end{pmatrix} = \begin{pmatrix} Rate\ of\ flow\ of \\ microorganims \\ into\ the\ system \\ boundary \end{pmatrix} - \begin{pmatrix} Rate\ of\ flow\ of \\ microorganisms \\ out\ of\ the\ system \\ boundary \end{pmatrix} + \begin{pmatrix} Net\ growth\ of \\ microorganisms \\ within\ the\ system \\ boundary \end{pmatrix}$$

Simplified word statement:

Accumulation = Inflow − Outlfow + Net Growth   **Equation C-1**

Symbolic kinetic representation:

$$\frac{dX}{dt}V_r = QX_o - QX + V_r r_g'$$ **Equation C-2**

Where dX/dt = rate of change of microorganism concentration in the reactor measured in terms of mass (volatile suspended solids), mass VSS/unit volume. time

V_r = reactor volume

Q = flowrate, volume/time

X_o = concentration of microorganism in influent, mass VSS/unit volume

X = concentration of microorganisms in the reactor, mass VSS/unit volume

And that after reaching steady state conditions in the reactor dX/dt =0

r_g' = net rate of microorganism growth, mass VSS/unit volume. time

Where μ_m = maximum specific growth rate, time^{-1}

The solution of the design equation is accomplished after combining the above mathematical expressions with the kinetic expression of biological growth in the bacterial culture within the reactor, which takes into account the rate of growth of bacterial cells and the rate of endogenous decay.

$$\frac{Q}{V_r} = \frac{1}{\theta} = \frac{\mu_m S}{K_s + S} - k_d$$ **Equation C-3**

Optical Management of Photosynthesis to Increase Efficiency of Hydrogen Production from Sugar-Processing Waste Water

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Abstract

In sugar processing, during the conversion of molasses into ethanol, a lot of waste water with high organic loading is discharged. Conventionally, simple methods, such as multi-stage lagoon treatment, are used to treat these waste waters. However, open air treatment generates very unpleasant odors, which negatively impact the surrounding environment. Furthermore, high concentrations of methane, carbon dioxide, and other gases are emitted into the atmosphere by lagoon treatment, which can influence global warming. In view of this situation, experiments to generate hydrogen gas, i.e., hydrogen energy, using photosynthesizing bacteria, were undertaken to process the high organic waste water discharged from sugar processing. In laboratory scale experiments, red non-sulfur photosynthesis bacteria (from the Rhodospirillaceae Family) were chosen as the photosynthesis bacteria. However, since the conversion efficiency from organic matter to hydrogen gas is poor, it was necessary to optically reinforce the red wavelength band which the bacteria use. In this research, as a result of performing experiments to improve the hydrogen manufacturing efficiency by photosynthesis bacteria, the following conclusions were reached: The available light by a wavelength conversion network was convertible for the suitable wavelength band for photosynthesis. Moreover, the knowledge for exploiting optical energy more efficiently was able to be acquired by reusing the light which usually passes by an optical amplification chip. Although the hydrogen gas obtained by experiment was 10 to 40% of theoretical, the validity of the photoactivating method was confirmed by this work.

Objectives

Sugar production worldwide since about 2000 is about 133 million tons, and molasses is about 48 million tons. Generally about 20 to 50% of molasses is produced from raw sugar production. Major producing areas of molasses are the EU countries, Asian nations, the United States and Brazil. The molasses trade volume in the world is about 16% of the total molasses produced. The main uses of molasses are for the fermentation industry to produce ethanol and as feed for livestock. The added value of molasses is low. Thus, experimental examination for the purpose of raising the value of molasses as a material for the production of hydrogen gas, a clean energy source, was undertaken. The objective of this research was to produce hydrogen gas with photosynthetic bacteria, using molasses organic waste water as the substrate and solar energy as the energetic source. Hydrogen production from waste water using solar energy and photosynthetic bacteria would have a very favorable environmental impact, both because of the production method and in the products.

Theoretical Background

(1) Photosynthesis bacteria

Photosynthesis bacteria grow in an underwater anaerobic environment, and perform a type of photosynthesis that does not generate oxygen. Growth environment differs in the algae, the blue algae, etc. Photosynthesis bacteria consist of four families, the Rhodospirillaceae, Chromatiaceae, Chlorobiaceae, and Chloroflexaceae. In this work, Rhodospirillaceae (purple non-sulfur bacteria) were used. The bacteria in this family carry out heterotrophic nutrition-growth photosynthesis (photo-heterotrophic growth), using various organic materials as the carbon source, and photosynthesis, with hydrogen as the by product. Therefore, these are able to grow bacteria in the anaerobic water layer containing many organic materials. The purple non-sulfur bacteria exhibit a perfect tricarboxylic acid cycle, and can use various organic sources. Unlike algae, photosynthesis bacteria do not perform photodegradation of water, but perform optical hydrogen generation by making various organic compounds into reducing power. Photosynthesis bacteria originate in the permeability of a metabolism system and a film etc., and the sources of carbon used according to the kind of bacillus differ.

(2) Hydrogen production

The enzyme which participates in generating and use of molecular hydrogen by photosynthesis bacteria is considered in two, hydrogenase and nitrogenase. Ferredoxin [worked type electronic conveyance object in the thylakoid membrane of a chloroplast] is displayed as Fd.

A)   Hydrogenase: $2H^+$ + Reduction type Fd ---> H_2 + Oxidation type Fd

B) Nitrogenase (dinitrogen fixation enzyme):
$2H^+$ + Returned type Fd (+ATP->ADP)?---> H2+ The reaction of oxidized type Fd(2)

Reaction B) generates hydrogen depending on hydrolysis of ATP.

Experimental

The equipment used for the experiment is shown in Table 1. The culture-medium composition for the photosynthesis bacteria is shown in Table 2.

Table 1. Equipment.

Equipment	Manufacturer
Burker Turk hemacytometer	#1743 (Erma Co., Tokyo)
Gas chromatograph	GC-SAIT (Shimadzu)
Spectrophotometer	DNA/RNA/Protein Analyzer (Shimadzu)
Microscope (Max x7000)	Hi-Scope Advanced KH-3000 (HIROX Co.)

Table 2. Culture-medium composition.

Medium Component	Volume (per 1000 ml)
NH_4Cl (Wako Junyaku Co.)	0.625 g
$NaHCO_3$ (Junsei Chemical Co.	0.625 g
CH_3COONa (Junsei Chemical Co.)	0.625
NaCl (Junsei Chemical Co.)	0.625 g
K_2HPO_4 (Wako Junyaku Co.)	0.125 g
$MgSO_4 . 7H_2O$) (Wako Junyaku Co.)	0.125 g
$HOOCCHOHCH_2COOH$ (Wako Junyaku Co.)	0.15625 g
$HOOC(CH_2)_2CH(NH_2)COOH$ (Wako Junyaku Co.)	0.15625 g
Distilled water	To volume

The photosynthesis bacteria were mainly Rhodospirillaceae and Rhodopseudomonas species. The concentration of photosynthesizing bacilli was measured by a calibration curve based on a measured absorption value. Glucose was measured colorimetrically. Measurement of ethylene, acetylene, and hydrogen was performed by gas chromatography. Figure 1 shows the general experimental set-up for growing the bacteria.

The hydrogen gas generated in the reaction vessel was brought into the external gas collection port, and was periodically measured. In order to maintain anaerobiosis in the vessel, the photosynthesis-bacteria concentration in the reaction vessel was measured at the time of the start of an experiment, and at the end. The bacterial concentration in a reaction vessel could also be roughly estimated by observing the red color change of the medium from the exterior. The relation of the nitrogenase activity and the hydrogen gas in connection with the photosynthesis was also measured.

Next, experimental research was done using a wavelength conversion network and optical reinforcement material for the purpose of improvement in the hydrogen manufacturing efficiency by photosynthesis. A wavelength conversion network is the material for using the ingredient of wavelength effective in photosynthesis alternatively among the wavelength contained in sunrays. Moreover, optical reinforcement material is the material for reinforcing artificially optical intensity required for hydrogen production of photosynthesis bacteria by reflecting repeatedly the light on which it was projected from the outside in the inside of a reaction layer.

Figure 2 shows the appearance of a wavelength conversion network and optical reinforcement material. The reaction vessel used for the experiment is glass, and the wavelength conversion network was twisted outside. The inside of a reaction vessel was made to diffuse optical reinforcement material, such as equipment used for experiment culture medium of photosynthesis bacteria.
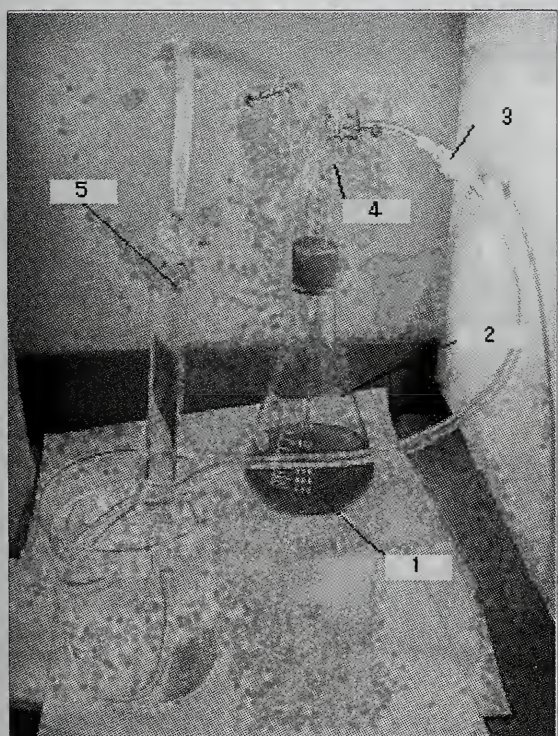
Figure 1. Experimental apparatus.
1-photosynthesis-bacteria culture medium;
2-cultivation tub; 3-adverse current
prevention valve; 4-substrate entrance
slot; 5-gas collection pipe.

Figure 2. Optical reinforcement materials.

Results and Discussion

Measurement of Nitrogenase Activity

Generally both hydrogen generation and nitrogenase activity in purple non-sulfur bacteria are dependent on optical intensity. Since nitrogenase converts acetylene to ethylene, we investigated the changes in acetylene and ethylene. Hydrogen generating filled the gaseous layer with argon, and mixed about 20% of acetylene. The ethylene concentration generated in the gaseous layer was measured after 24 hours. The ethylene concentration measured by gas chromatography is shown in Table 3. The change to ethylene from the acetylene gas in which the nitrogenase activity in photosynthesis is acetylene concentration had become zero, ethylene was undetectable in 24 hours after an experiment start. The fact that acetylene disappeared even when ethylene was undetectable shows anyway that acetylene was consumed by the photosynthesis bacteria. It was also possible that there is no relation more direct than ethylene was not able to detect acetylene in spite of having accepted generating of hydrogen in ethylene between photosynthesis and nitrogenase activity, or that the method of measurement was inadequate.

Table 3. Concentration of acetylene and ethylene gas in the bottle.

Processing time, hr.	Acetylene Conc. (%)	Ethylene Conc.
0	20.0	0
24	0.4	0
48	0	0
72	0	0
96	0	0
120	0	0

Basic Experiment with Glucose

The relation between glucose consumption and bacterial concentration is shown in Figure 3. While the amount of glucose consumption increases over time, it is noted that the amount of photosynthesis bacteria decreased less rapidly with time. The rate of glucose consumption tended to increase over time. It seemed that the amount of photosynthesis bacteria decreased gently as the quantity of the glucose which is a substrate decreased since this experiment was a batch examination. Input substrate concentration was judged to be inadequate (poor nutrition) compared to the amount of bacteria which existed in the reaction vessel.

Figure 4 shows the relation between the amount of glucose consumed, and the generated hydrogen gas. The amount of hydrogen gas generated per unit weight of glucose consumed based on reaction time is shown. Peak hydrogen generation per unit of substrate occurred at about 70 hours. Per unit of hydrogen gas, the amount of gas generation increased at the reduction stage of the number of photosynthesis bacteria, and began to decrease from the time of the number of photosynthesis

bacteria started to increase. From this result, it was predicted that there is an optimal quantitative relation between the number of photosynthesis bacteria in a system, and the substrate.

Fig.3 Relation between Glucose and Bacteria

Fig.4 Relationship between procrssing time and Generated Hydrogen per Glucose Unit(mM)
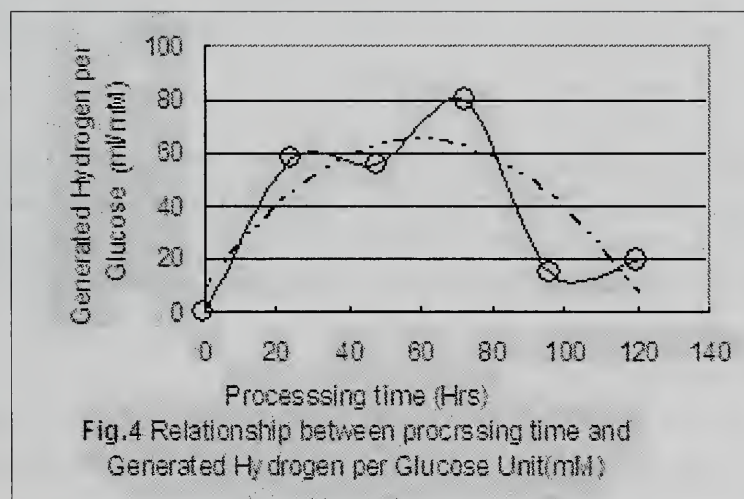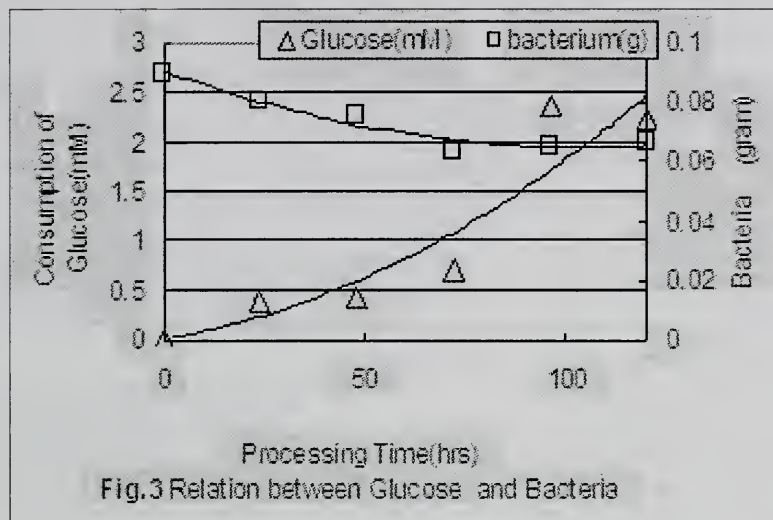
Improvement in Hydrogen Manufacturing Efficiency

The quantity of hydrogen produced by photosynthetic bacteria is influenced by various factors, such as pH, temperature, optical intensity, nutritional factors and substrate ingredients (carbon source). In this research, the effect exerted by reinforcement of ultraviolet-rays intensity using molasses as a substrate on the increase in hydrogen production was investigated. The following two methods were adopted as a method of raising the optical intensity of ultraviolet rays. (1) Make an effective light increase by twisting the network which has infrared selectivity in the outline of a reaction vessel. (2) Raise the optical use efficiency within a tub by arranging reflective material on the inside of a reaction vessel, causing it to carry out diffused reflection of the light from the outside. Generally, although penetration of the light into a reaction vessel is only one time, by this method, it is reflected repeatedly and repetitious use of the light is carried out. Table 4 shows the composition of the molasses used for the experiment. Since photosynthesis bacteria growth might be inhibited by the

presence of ammonia in molasses, it was thought that a substrate with a high nitrogen concentration was negative factor for efficient hydrogen generation. It aimed at predicting improvement in hydrogen manufacturing by conducting a check experiment about multiplication of photosynthesis bacteria this time.

Table 4. Componential analysis result of the molasses

Component	Concentration
Protein	2.5 g/100 g
Total Carbohydrate	56.0 g/100 g
Of which is sugars	56.0 g/100 g
Fat	Trace
Fiber	Trace
Sodium	0.1 g/100 g
Chemical Oxygen Demand	237.5 g/L
Total Nitrogen	12.8 g/L

The characteristics of the wavelength conversion network is shown in Figure 5. The fluorescence peak is near 600 nm which is useful for enhancing photosynthesis. The relation between radiation illumination and fluorescence is shown in Figure 6. It shows that optical intensity is increased in the 500 nm to 600 nm range for the wavelength of light.

Fig.5 Characteristics of Absorbance and Fluorescence on the Light Selective Net.

Fig.6 Relationships between Irradiance and Wavelength (Spectrum)

Fig 7. Increment from blank test by this method is showed
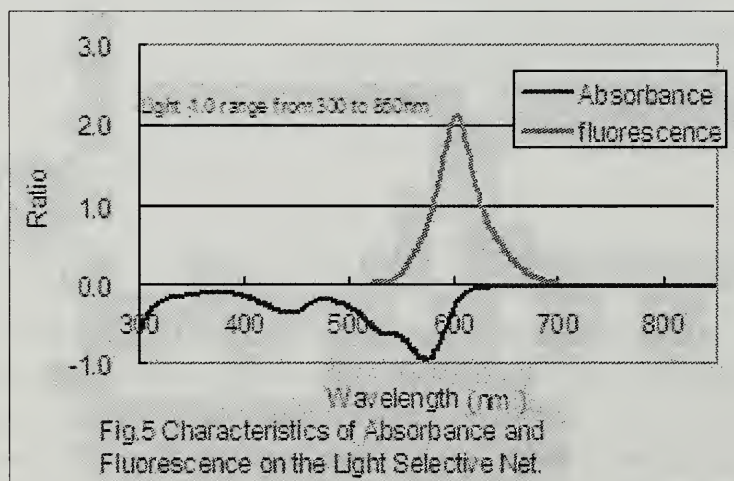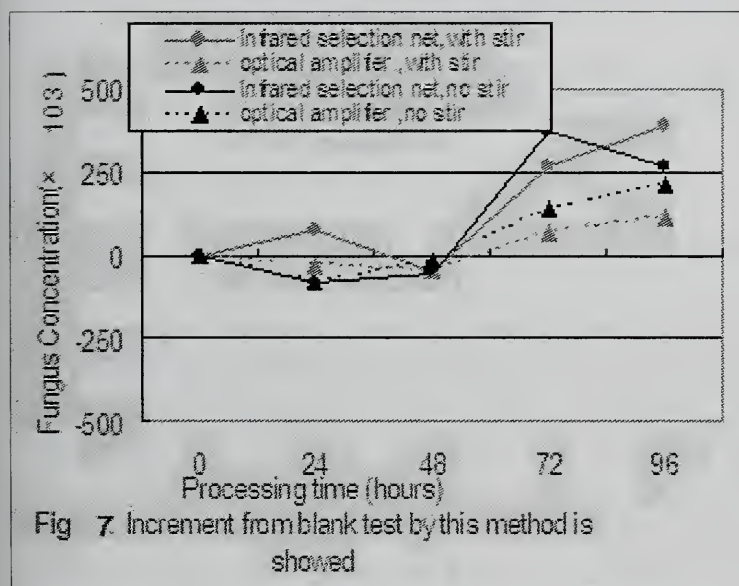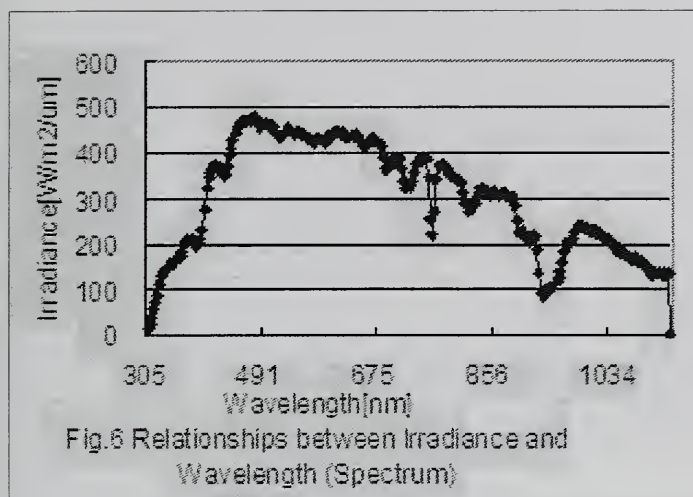
As shown in Figure 7, the photosynthesis enhancing effect at the time of using a wavelength conversion network and an optical amplification chip was checked by investigating the multiplication situation of photosynthesis bacteria. The effect was notably accepted for the wavelength conversion network and the optical amplification chip about 60 hours after a processing start. The cause which was in discovery of an effect was able to consider that the influence and the effect of change-of-generation time of photosynthesis bacteria were the material of the type which permeates gradually. When compared with the blank which was carried out with no optical control, the increase in the number of bacteria between 100×10^3 and 400×10^3 was checked. Since the increase in the number of photosynthesis bacteria was checked by providing the means for strengthening light, naturally an increase in the hydrogen gas produced with bacteria is expected. However, the verification about this anticipation will be reported in a future experimental result. The increase in the amount of multiplication of photosynthesis bacteria was checked about 60 hours after the reaction start. It seems that the increase in bacterial count is also correlated with the increase in the hydrogen gas generated.

Conclusion

Since the energy used in hydrogen production by photosynthesis bacteria is sunlight, and the sources of carbon are wastes of organic nature, the potential is great. However, since the conversion efficiency from an organic substrate to hydrogen gas is poor, it has been doubtful of utilization. In this research, as a result of performing experimental research about improvement in the hydrogen manufacturing efficiency by photosynthesis bacteria, the following conclusions were obtained. (1) The available light by a wavelength conversion net was convertible for the suitable photosynthetic wavelength range. (2) The knowledge for exploiting optical energy more efficiently was acquired by winding again the light which usually passes by an optical intensity amplification chip, by using a wavelength conversion net and an optical intensity amplification chip.

This work reports the fundamental data about improving hydrogen manufacturing efficiency by photosynthesis bacteria. Further detailed experimental work is required in order to develop a commercially utilizable system.

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Control of Surface Properties and Efficiency of Some Antifoams Used in Beet Sugar Processing

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Abstract

In the sugar factory, foam formation occurs at all stages of processing, beginning with beet washing. One of the main problem ares in this respect is that of diffusion extraction. Numerous problems are associated with foam formation, such as extractor clogging, extraction yield decrease and sugar loss due to vat overflow. To prevent these problems, antifoams are injected. However, the formulas of such technical aids are complex and generally patented. Thus, the user of antifoams has to use the services of suppliers who keep secret their formula. In this work we have compared different formulas of antifoams by studying their mechanism of action and optimizing the efficient doses.

Relative efficacy of six samples of antifoams was determined as a function of concentration and temperature. It was shown that efficacy increases with concentration and shows a threshold. The effect of temperature resides in the need that antifoams are added at a temperature above the turbidity point. Likewise, antifoams were classified as a function of their hydrophobicity and efficacy at normal temperature. It was shown that efficacy increases with hydrophobicity. The more efficient an antifoam, the slower is its adsorption at the air/liquid interface. A mechanism for trapping of juice surface active molecules by antifoams is proposed.

Finally, adsorption kinetics was studied at high temperature for antifoams quickly adsorbed at normal temperature. It was found that increasing the temperature facilitates the adsorption of antifoams at the interface. Such an adsorption is the more important the more efficient the antifoam is at normal temperature. Decrease in activation energy for the adsorption of antifoam from subsurface to interface was proposed as a possible explanation of antifoam behavior at interfaces.

Introduction

In sugar factories, foam is formed at almost all stages of processing, especially during juice extraction. This is due to surface active molecules (saponins, proteins, peptides, etc.) extracted from beet on the one hand, and to the agitation and pumping which incorporates air in the juice on the other hand (1).

Different drawbacks are linked to foam formation, like diffuser clogging, decrease in sugar extraction yield, overflow in tanks which induces appreciable sugar loss (2). To prevent foam formation, the general rule is the injection of antifoam products. Different patents describe these products (3, 4, 5, 6, 7, 8) which are generally composed of copolymers of ethylene and propylene oxides (70%) and mineral oil (30%). The ratio of ethylene/propylene polymers varies with antifoam formula and application. In addition, the polymer may or may not be esterified with fatty acids or polyols. Other minor constituents may also be found in the formula such as colorants or emulsifiers. To summarize, the antifoams used in sugar factories are complex formulations, most of the time protected by patents, and composition is difficult to know in detail. Despite the lack of information, the users in factories are more or less obliged to use commercial formulas and their reaction in cases of foam increase is to increase the dose of antifoam or to use new, expensive formulas recommended by suppliers. However, the tendency now is to use fewer and fewer technical aids to be environmentally friendly.

In this paper, we report results of an optimization study of antifoam dose for six commercial formulas used in sugar beet diffusers. We also have determined surface properties and cloud points for the six studied antifoams and propose a mechanism of action of the antifoams based on the comparison of their properties and efficacy.

Materials & Methods

Foaming control solution: A diffusion juice free from antifoam was used as reference material for foaming studies. The juice (16° Brix) was sampled during the 2005 beet sugar campaign at a partner factory and preserved at 4° C after adding azide as a preservative.

Antifoam samples: The commercial antifoam samples used in this work were supplied by the two major suppliers of technical aids to the French beet sugar factories. We have named them AM1, AM2, AM3, AM4, AM5 and AM6.

For all antifoams used, a sample of the polymers constituting it was supplied. The following information on the composition of antifoams was available:

AM1: Ethylene and propylene copolymer oxides esterified with a vegetal fatty acid mixed with mineral oil.

AM2: Polyether-polyol esterified with a vegetal fatty acid mixed with mineral oil.

AM3: Ethylene-propylene copolymer oxide esterified with fatty acid mixed with mineral oil.

AM4: Copolymers esterified with a plant fatty acid mixed with vegetal oil and mineral oil.

AM5: Copolymers of ethylene and propylene oxides esterified with vegetal fatty acid mixed with mineral oil.

AM6: Mixture of polyalkoxyether and mineral oil.

Method of control of antifoam efficiency:

Foaming at ambient temperature: Experiments of foaming at ambient temperature (22 +/- 2° C) in presence or not of antifoam were performed using a Foam Scan apparatus (IT Concept, Lyon, France). Foam was obtained by nitrogen bubbling in the foaming solution. A constant flow of Nitrogen (15 mL/min) was fed through the sintered glass bottom of a column (L = 30 cm; Diameter = 3 cm) containing 15 mL of juice. The column backlighted white a light source on one side and foam pictures were taken with a CCD camera on the opposite side. Pictures were taken each second, digitized and analyzed by a computer which delivered the height of foam as a function of time.

Foaming at high temperature: Three antifoams (AM4; AM5; AM6) found to be less efficient at normal temperature were analysed at 70° C. Sample AM6 was also analysed at 85° C. The device used at high temperature was the Jet Scan device (IT Concept, Lyon, France) where foaming was obtained by shearing in a temperature controlled glass column (L = 50 cm; Diameter = 10 cm.). The foaming solution was pumped into the column through a loop at high speed (3.5 m/s). The vertical stream of liquid allows the formation of foam by shearing. The column was backlighted and pictures were taken each second, digitized and treated by software to give the variation of foam volume as a function of time. Treatment of data was similar to that of the foam scan.

Relative efficacy of antifoams: Relative efficacy (R.E.) of antifoams is expressed in terms of percentage of foam inhibited by the addition of a certain concentration of added antifoam. It is calculated as follows:

$$R.E. = (V_{max} \, p.j. - V_{max} \, J.af)/ V_{max} \, p.j.$$

where V_{max} p.j. is the maximum volume of foam obtained in pure juice and V_{max} j. af is the maximum volume of foam obtained in juice with added antifoam at a given concentration in the same conditions of temperature and foaming.

Cloud points: Cloud points of copolymers corresponding to the formula of the different antifoams were determined by the measurement of absorbance at 420 nm at temperatures varied from 20-90° C. Measurements were made for solutions containing 1 mL of copolymer and 99 mL of double-distilled water. Absorbance was measured with a Shimadzu UV-2101 spectrophotometer. Sample temperature was controlled with a Polyscience 9110 –RH thermostatted bath. Rate of increase of temperature was 2° C/min.

Contact angles (θ): Measurement of contact angles of antifoam samples with a hydrophobic surface (Teflon) was made at 22° C. The aim of such measurements was to classify antifoams on the basis of their hydrophobicity. This measurement consists in spreading a drop of antifoam on a perfectly smooth hydrophobic surface. The more the sample has affinity for the hydrophobic surface, the lower the angle between the tangent to the first contact angle of the drop and the

horizontal plane. Measurements were made with a drop tensiometer (IT Concept, Lyon, France). A small drop (2 µL) of antifoam at the end of a syringe was deposited on the Teflon surface. A CCD camera took pictures each second and transferred them to a computer with appropriate software (Old drop, IT Concept) which determined contact angles (θ) from image analysis.

Kinetics of adsorption of antifoam at air/water interface: Measurement of surface tension was made using a bubble tensiometer (IT Concept, Lyon). A description of the method was reported by Benjamins *et al.* (9). A bubble of 2 µL volume was formed at the extremity of a syringe in the middle of a sample (20 mL) of antifoam. The shape of the bubble was recorded with a CCD camera and images analysed with dedicated software (Old drop, IT Concept). Surface tension was determined from the analysis of the bubble profile by application of the Laplace equation:

$$1/x/dx(x\sin\theta) = 2b - cz$$

Where x and z are coordinates of different points in the bubble profile in a Cartesian system, θ is the angle of the tangent to the bubble profile with the x axis, and b is the curvature radius at the bubble apex (Figures 1 and 2); c is a capillary constant ($c = g\rho/\sigma$) with ρ the difference in density between juice and air; g gravitation acceleration and σ the interfacial tension. A 5 mL sample of diffusion juice was mixed with 20 ppm (v/v) of antifoam and used in surface tension measurement. Surface tension was measured every 30 seconds after bubble formation. To eliminate any suspended matter in the juice, 2 bubbles were formed and destroyed prior to data collection. Another method of elimination of suspended matter applied was the centrifugation of samples for 10 min at 10,000 rpm at ambient temperature.
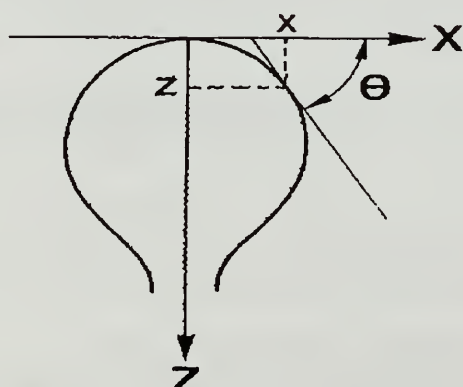
Figure 1. Bubble profile analyzed by
the Laplace equation.
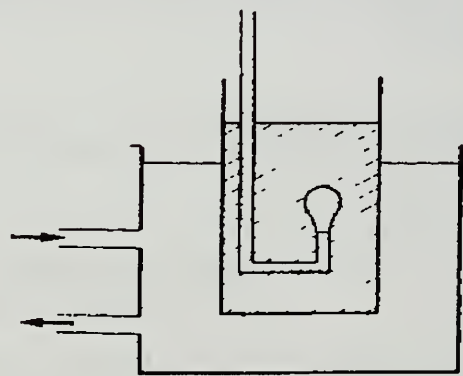
Figure 2. Controlled temperature cell
for surface tension analysis.

Analysis was performed at 22° C +/- 0.5° C in a first series of determinations for all antifoam samples and at 70° C in a second series of measurements for antifoams AM4, AM5, and AM6. Temperature control was maintained with circulation of water from a thermostatted bath, as shown in Figure 2. Densities of juice samples were measured with Anton Paar DMA 4500/5000 and the values were 1.058 and 1.033 respectively at 22° C and 70° C.

Results and Discussion

Efficacy of antifoams as a function of concentration:

R.E. at ambient temperature: Relative efficacy was determined for all six samples of antifoams at ambient temperature in the range of concentrations from 0-100 ppm with the aim of determining the optimal concentration. Results are reported in Figure 3. A difference in efficiency was observed as the concentration of antifoam varied. For example, a dose of 10 ppm of AM1 eliminated 99% of foam while only 20% foam was eliminated by the use of the same concentration of AM6. Figure 3 also shows that for a certain concentration, which is specific for each antifoam, it is useless to increase the dose of antifoam as no additional efficacy (foam inhibition) is obtained.
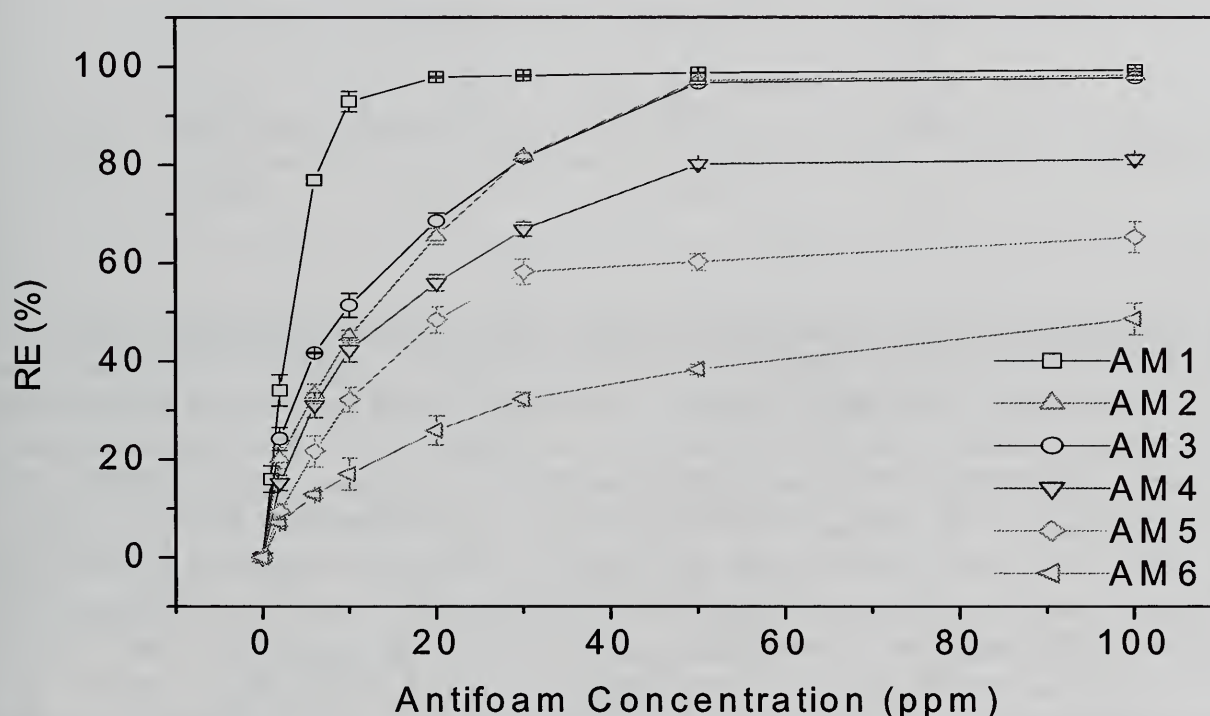
Figure 3. Relative efficacy of antifoams as a function of concentration at 22°C

RE at high temperature: Three antifoams (AM4, AM5, AM6) found to be relatively less efficient at ambient temperature were analysed at 70° C for 3 concentrations: 10, 50 and 100 ppm as summarized in Table 1. It is shown in Table 1 that the increase of temperature from 22° C to 70° C noticeably improved the efficiency of AM4 and AM5. Therefore, a concentration of 10 ppm yielded improvements of 25.68% and 24% respectively for AM4 and AM5. With 50 and 100 ppm, improvement of efficiency was less remarkable (2.13% for AM4 and 9.94% for AM5). It should also be noted that at 70° C foam is formed by shearing while it is formed by bubbling at ambient temperature.

For AM6, the increase in efficacy was significant as temperature increased from 22° C to 70° C. However, the values obtained at 70° C were not satisfactory compared to AM4 and AM5. It was necessary to increase the temperature to 85°C for AM6.

Table 1. RE of AM4, AM5 and AM6 antifoams at different temperatures and concentrations

Antifoam	Concentration (ppm)	RE at 22±2°C (%)	RE at 70 ± 2°C (%)	RE at 85 ±2°C (%)
AM4	10	42.32 ± 2	68 ± 2	-
	50	80.12 ± 1	82.25 ± 1	-
	100	81.06 ± 1	84± 1	-
AM5	10	32.18 ± 2	56.2 ± 2	-
	50	60.21 ± 1.5	70.15 ± 1	-
	100	65.26 ± 3	76.32 ± 1	-
AM6	10	17.01 ± 3	43.1 ± 2	55.81 ± 2
	50	38.31 ± 2	55.8 ± 1.5	75.64 ± 1
	100	48. 61 ± 2	60 ± 2	79.74 ± 1.5

Antifoam efficacy as a function of concentration after numerous cycles of foaming: Efficacy of AM6 after 3 successive cycles of foaming was determined at 70° C and 85° C. A foaming cycle consisted of 300 seconds of shearing (pump circulation) to form the foam followed by 300 seconds of rest to achieve complete drainage of the formed foam. Four concentrations of antifoam were used (0, 10, 50, 100 ppm) at 70° C and 85°C. Results are shown in Figures 5 and 6. We observed that in the absence of antifoam, foam volume increased after each cycle of foaming. This may be due to the fact that the shearing in the second and third cycles created more and more interfaces between air and liquid as schematically represented in Figure 4. The newly formed interface is less concentrated in surface active molecules than that before the second or third cycle of shearing. To obtain a homogeneous repartition of surface active molecules at the interface, an inflow of surface active monomers from the region surrounding interfaces is necessary. Such a contribution of monomers ruptures the equilibrium between micellar and monomeric forms (10) of antifoam in the solution. Therefore micelles of surface active molecules constitute a reservoir feeding the interface. If interfaces are regularly fed in monomers, there is more and more foam after the second and third shearing cycles (Figure 4).

It may be observed in Figure 5 that for the first cycle of foaming, increased antifoam concentration gradually increased the efficacy (foam volume reduction). However, in the second and third cycles, maximum foam volume was obtained with 50 ppm AM6 at 70° C is situated above that obtained with 10 ppm. This phenomenon is not observed when temperature is raised to 85° C (Figure 6).
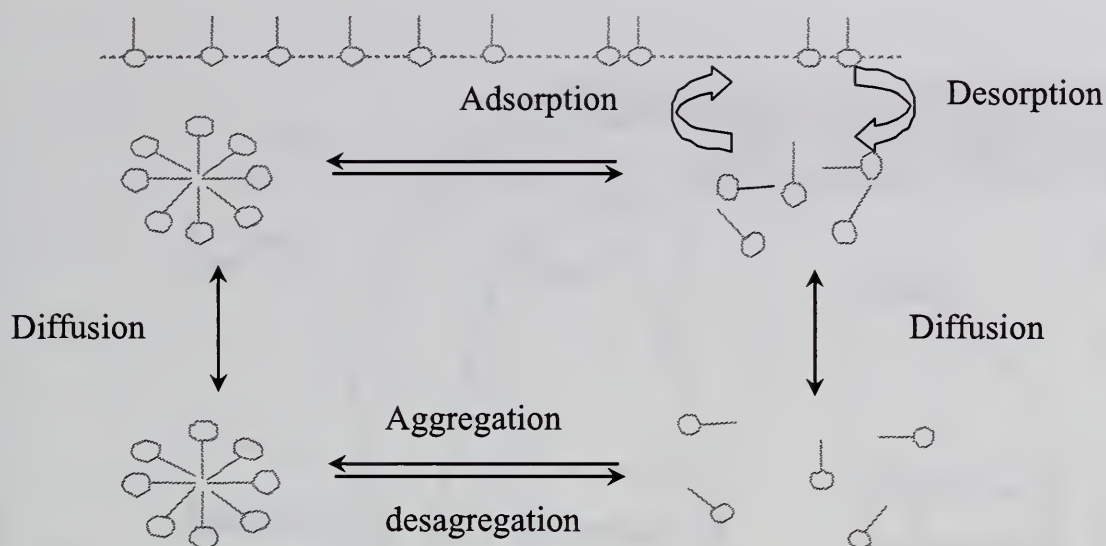
Figure 4. Schematic representation of monomer-micelle equilibrium in a foaming system (10).
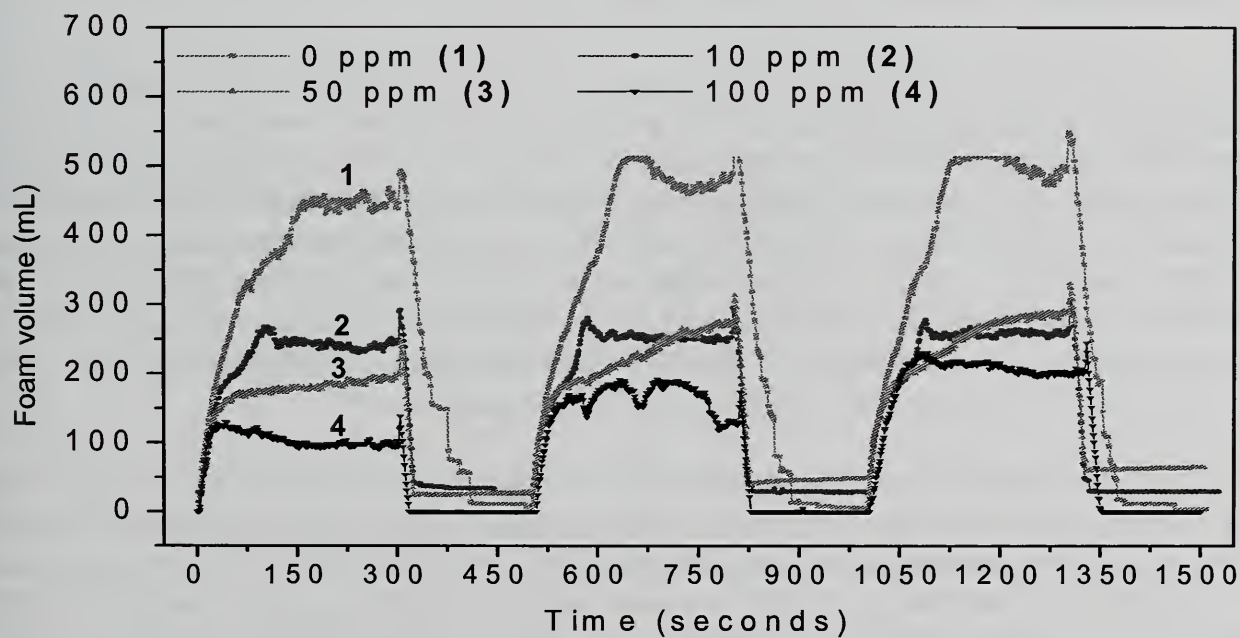
Figure 5. Volume of foam obtained without (0 ppm) and with 10, 50, or 100 ppm of AM6 antifoam at 70° C and 3 successive cycles of foaming.
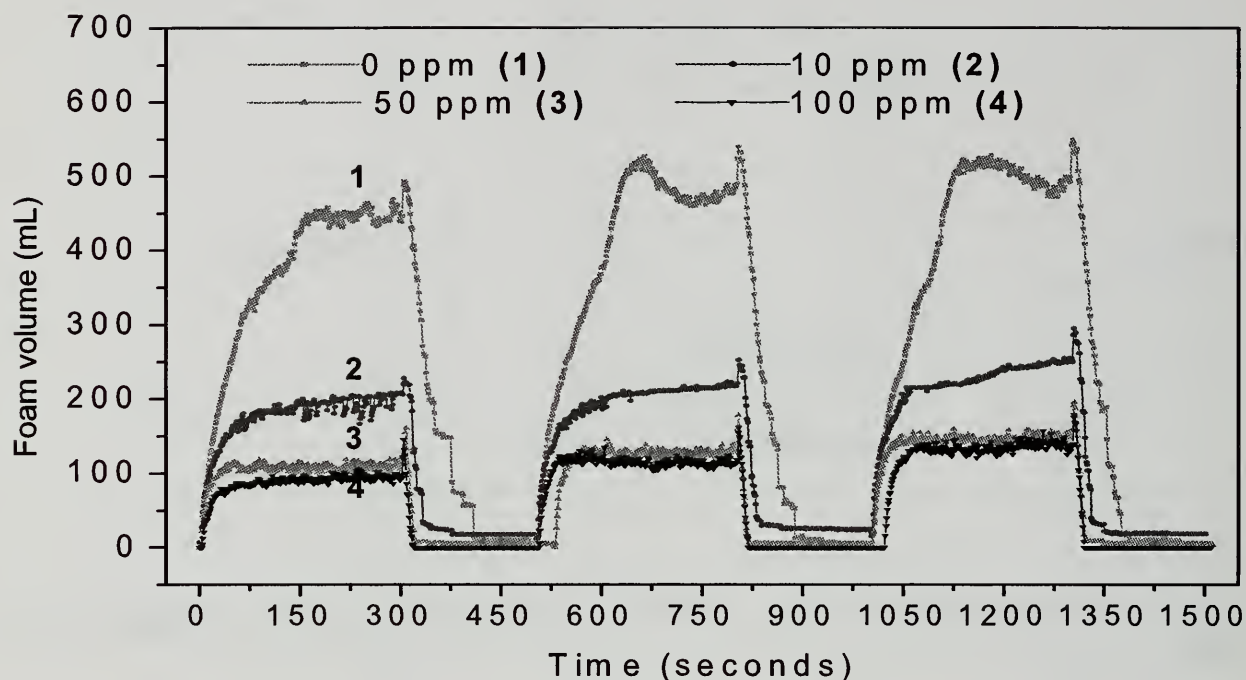
Figure 6. Volume of foam obtained without (0 ppm) and with 10, 50 or 100 ppm of AM6 antifoam at 85° C after 3 successive cycles of foaming

Cloud points and antifoam efficacy: Cloud points of copolymers corresponding to the six antifoams were measured. Results are summarized in Figure 7. Two copolymers corresponding to antifoams AM2 and AM4 were called AM2-A, AM2-B and AM4-A and AM4-B, respectively. Observation of Figure 7 shows that cloud points for copolymers corresponding to antifoams AM1, AM2 and AM3, which are most efficient at low temperature, are situated at lower temperatures than those corresponding to AM4, AM5 or AM6. For this latter antifoam (AM6), it was not possible to obtain a sample of polymer from the supplier. Therefore, the cloud point reported in Figure 7 was measured on the whole AM6 formula.

The cloud point of an antifoam is, by definition, the temperature at which solubility is reversed. At a value below the cloud point, antifoam is water soluble. Above this temperature it becomes insoluble. At a molecular level, this change in solubility is due to the dehydration of polar heads. Such a breakage of low energy bonds between water and polar groups results in micelle formation. Hydrophobic interactions occur between surface active molecules, water is repelled and the product remains insoluble. Micelle formation corresponds to a minimum free energy of the system.
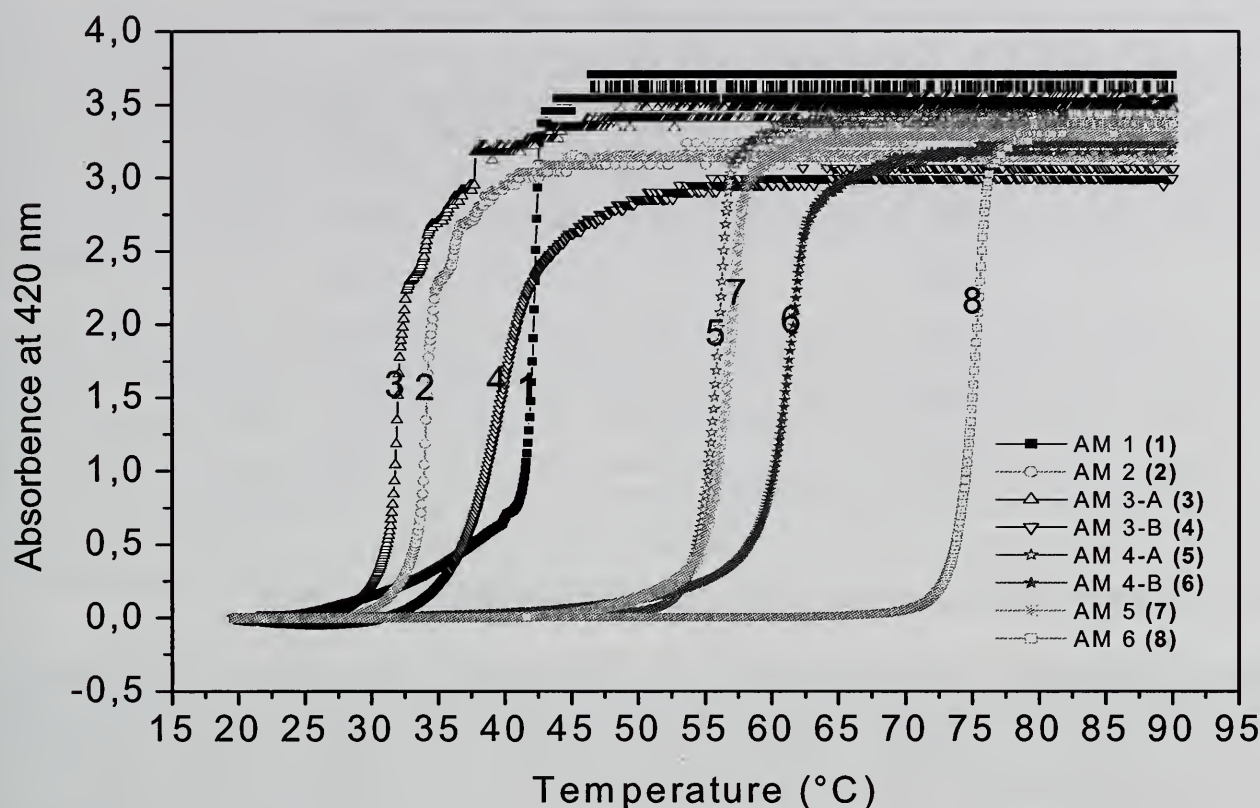
Figure 7. Cloud points of copolymers constituting the experimented antifoams.

For AM6, Figure 7 and Table 1 show that this antifoam does not show any cloud at ambient temperature, which gives a relatively low efficacy (R.E. = 38.31 +/-2% at 50 ppm). At 70° C, AM6 starts forming a slight cloud. Its efficacy is improved (R.E. = 55.8 +/-1.5% at 50 ppm). At 85° C, the cloud point is reached and efficacy is at its maximum (R.E. = 75.64 +/-1% at 50 ppm). On the other hand, as already mentioned, AM6 shows a loss of efficacy after repeated cycles of foaming at 70° C for a concentration of 50 ppm (Figure 5).   At 85° C, for the same concentration, efficacy loss is not observed (Figure 6). This is explained by the inhibition of transfer of juice surface active molecules to the freshly formed air/liquid interfaces. Two hypotheses may be proposed in this case: either the antifoam prevents the disintegration of juice surface active micelles, or it inhibits their diffusion from bulk solution to the vicinity of interface (Figure 4).

Foam hydrophobicity and efficacy: As antifoams are more efficient when they form clouds (micelles), their cloud point temperatures and efficacies vary with their composition. The property responsible for such variability is very likely the degree of hydrophobicity of antifoam which may be evaluated by HLB (Hydrophilic-Lipophilic Balance) or eventually the contact angle with a hydrophobic surface. A classification of the studied antifoams is proposed based on θ, the contact angle measured as described earlier (Table 2). Comparison of antifoam hydrophobicity and foam inhibition efficacy shows that the more hydrophobic the antifoam is, the higher its relative efficacy.

Table 2. Contact angle of the studied antifoams

Antifoam	Contact Angle θ (°)
AM1	46.75
AM2	49.27
AM3	59.63
AM4	60.34
AM5	65.94
AM6	66.64

Adsorption of antifoams at air/liquid interface and mechanism of action

At ambient temperature: The kinetics of antifoam adsorption at the interface was measured at ambient temperature. Air/liquid interface was obtained by the formation of a bubble of controlled size in the solution composed of diffusion juice mixed with 20 ppm antifoam. Adsorption kinetics was recorded each second for 30 seconds just after the formation of the bubble. This time (30 s) corresponds to the formation of the first adsorption layers of tensioactive molecules (from the juice and/or the antifoam) at the interface. Results are shown in Figure 8.
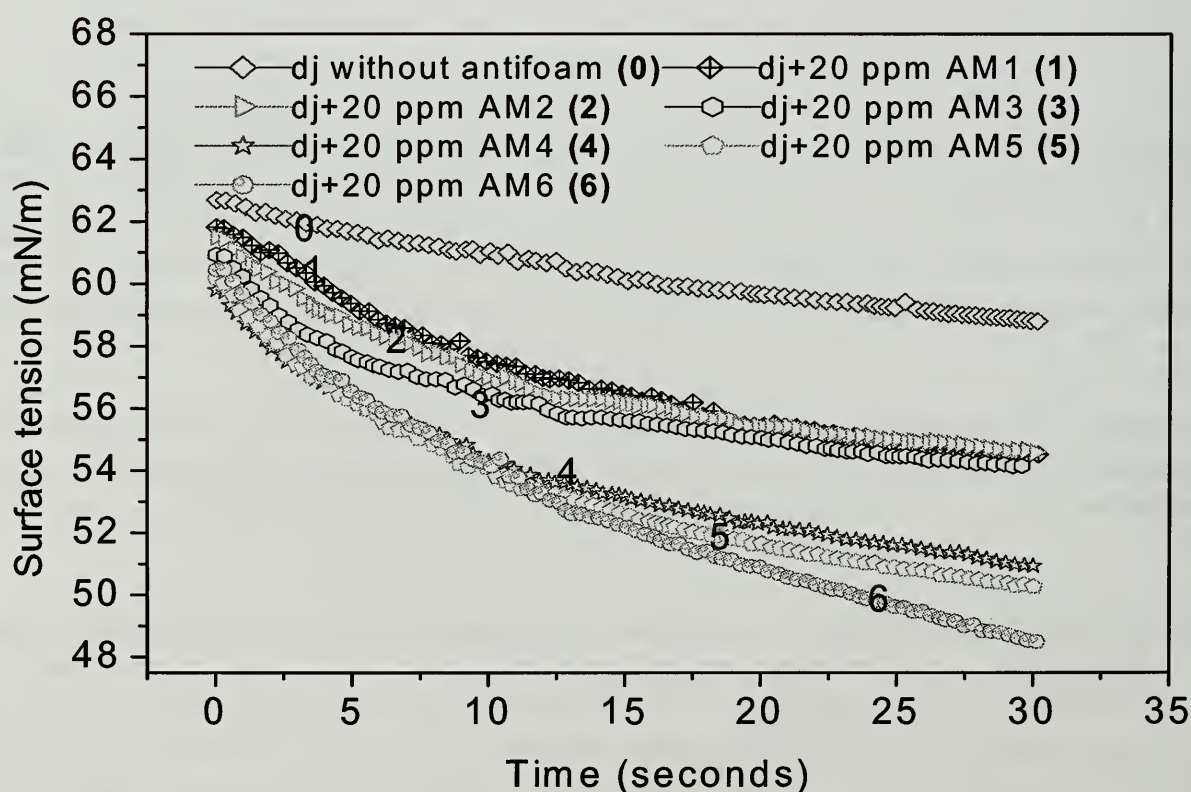
Figure 8. Evolution of surface tension as a function of time (adsorption kinetics).

It may be observed that surface tension decreased gradually as a function of time for all samples composed of diffusion juice without and with 20 ppm of each of the antifoams (Figure 8). To assert the pertinence of the measurement of surface tension, it should be recalled that the measured value results from 2 forces acting on the bubble: its weight and the surface tension. Weight tends to lengthen the bubble, and surface tension, on the contrary, tends to maintain a spherical shape. Whereas weight is constant, surface tension varies with the quantity of adsorbed molecules.

For juice free from added antifoam, the value of surface tension is around 63 mN/m, slightly higher than that reported by VanHook and Biggins (2). This might be due to the preparation of the juice sample which was centrifuged to eliminate suspended matter which may influence the measurement of surface tension. On the other hand, the slope of the surface tension decrease as a function of time was much weaker in the case of pure juice. Only juice surface active molecules are adsorbed. In the case of adsorption of both juice and antifoam surfactants, the slope changes and seems characteristic of each antifoam (Figure 8).

As the variation of surface tension as a function of time accounts for the adsorption on the bubble wall of the surface active molecules, the slopes of the tangents at the origin of the curves represented in Figure 8 were determined and are reported in Table 3. Comparison of surface tension curves slopes to the values of relative efficacies of the six antifoam samples at ambient temperature shows that the slower the adsorption of antifoam at the air/liquid interface is, the higher the efficacy of foam inhibition (Table 3). This means that efficacy of antifoam is linked to its staying a longer time in the solution rather than rapidly reaching the air/solution interface. This suggests a possible mechanism of action which consists in trapping the surface active molecules from diffusion juice by the antifoam and preventing them from forming foam.

Table 3. Slopes of tangents at the origin of curves representing surface tension evolution and relative efficacies of the different antifoam samples

Sample	Slope of tangent at origin of surface tension curve	R.E. (%)
Pure diffusion juice	- 0.210	----
AM1	- 0.626	97.82
AM2	- 0.665	65.42
AM3	- 0.669	68.58
AM4	- 0.749	55.97
AM5	- 0.887	48.40
AM6	- 0.957	25.89

At high temperature: Kinetics of adsorption of antifoams (AM4, AM5 and AM6) which are less efficient at ambient temperature was followed at 70° C as well as for juice free from antifoam. Results are reported together with that obtained at 22° C in Figure 9. It may be observed that for the juice free from antifoam, surface tension decreased rapidly at 70° C as compared to 22° C.

This is very likely due to the effect of temperature on the diffusion of surface active molecules as well as on the activation energy of adsorption at the interface which is reduced as temperature is increased. Moreover, Figure 9 also shows that the difference in surface tension between the values obtained at the 2 temperatures was much higher in the absence of antifoam as it is in their presence. This feature is also interpreted as an argument in favour of the trapping of surface active molecules from beet diffusion juice by antifoams, which act as inhibitors of foam promotion. In the case of samples containing antifoam, it should be noticed that the more efficient is the antifoam, the larger the difference between curves recorded at 70° C and 22° C. We recall that antifoams are in the form of micelles at 70° C and their behavior at the air/liquid interface is described as spreading rather than adsorption. A large difference between the two curves denotes an easier spreading of antifoam on the film of foam, which leads to a more important de-foaming effect.
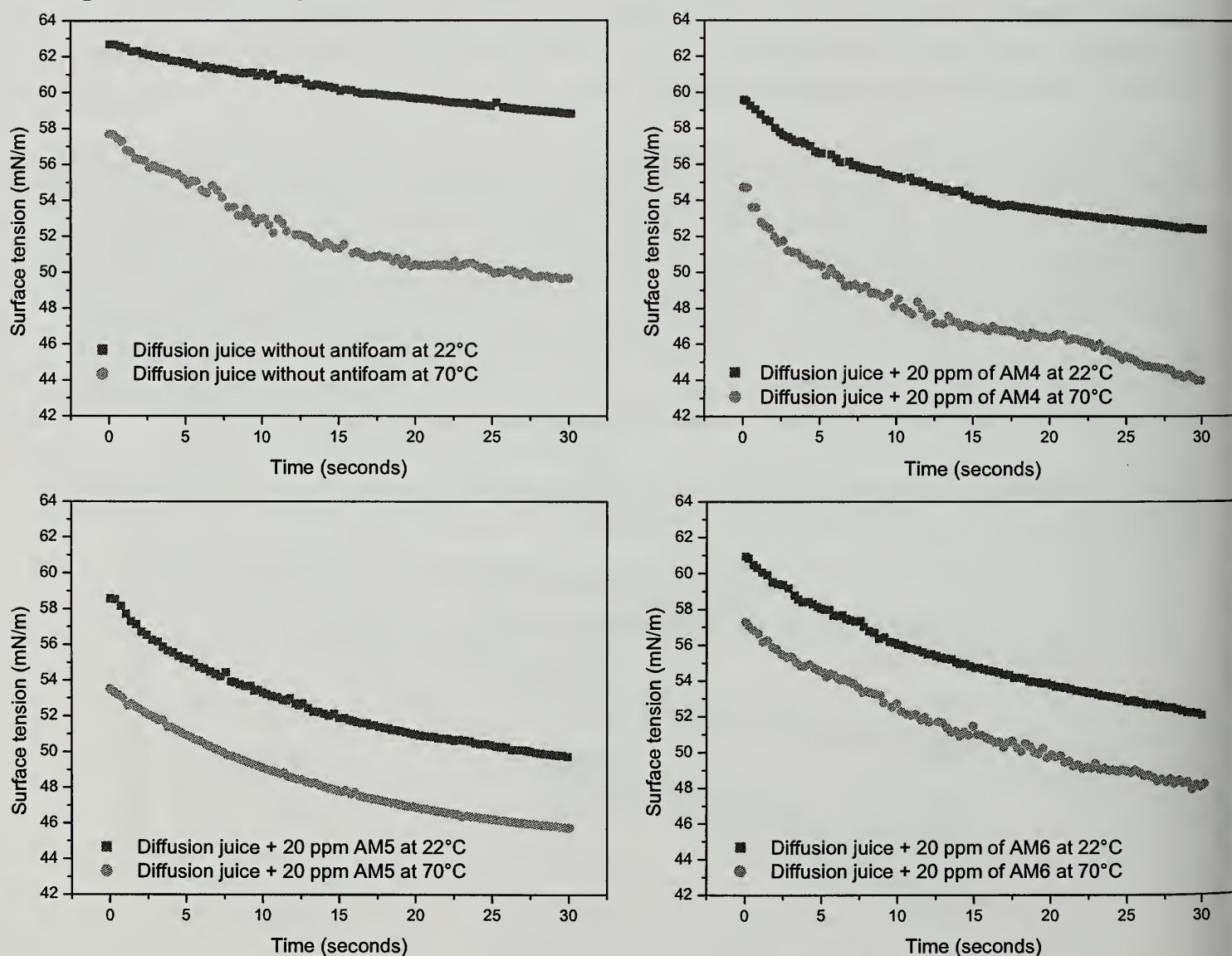
Figure 9. Evolution of surface tension in diffusion juice without and with AM4, AM5, and AM6 antifoam (20ppm) at 22 (blue) and 70°C (red)

Conclusion

From the experimental work presented in this paper, we may conclude that:

- The efficacy of antifoams, apart from their composition, mainly depends on two parameters: concentration, which shows a threshold value, and temperature, which allows to reach the cloud point.

- Depending on their localization in the foaming system, antifoams seem to proceed according to two different and complementary mechanisms of action:

  o In solution: Antifoams act to prevent foam formation. This is achieved by delaying the adsorption of surface active molecules at the air/liquid interface. The more hydrophobic the antifoam. the higher is this type of antifoaming effect.

  o Near the interface: Antifoams exert a de-foaming effect by spreading on the films of formed foam. As the hydrophobicity of the antifoam increases, the spreading becomes easier and the de-foaming more significant.

Acknowledgements

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Medium Density Fiberboard (MDF) Made from Sugarcane Bagasse Lignin and Fibers

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Introduction

Medium density fiberboard (MDF) is a wood-based material made from lignocellulosic fibers bonded together by synthetic resin under heat and pressure. It is one of the most widely used wood composites as a substitute to manufacture housing furniture. With the rapid development in the production and usage of MDF, the use of annual plant fibers in fiberboard is very advantageous. First of all, annual plants achieve a full maturity cycle in 12 month, while a tree takes years to grow. Secondly, deforestation is an environmental problem and could be limited by the use of annual plant instead of wood in the fiberboard industry. Among annual plants, bagasse is the solid lignocellulosic residue left after extraction of juice from sugarcane. A large part of bagasse is burnt for energy supply in a sugarcane factory, but it is also used to make paper pulp, board materials and composites. In boards, the existence of physical, chemical or physico-chemical interactions between resin and fibers is very important for the final product properties. The interactions between fibers and resin depend on the extension of the contact area and on the affinity between components. The latter can be intensified by chemical treatments applied to the surface of the fibers and/or by changing the resin formulation. Both possibilities were considered, e.g. lignin was used as a substitute for phenol in resin formulation and/or fibers were chemically modified. The chemical modification of sugarcane bagasse fibers was done by oxidation with ClO_2 and grafting furfuryl alcohol (FA). Fiberboards were made with sugarcane bagasse lignophenolic prepolymers and bagasse fibers.

Chemical Modification of Fibers

The mechanism of grafting of furfuryl alcohol (FA) onto oxidized fibers remains unknown; it might involve Diels-Alder addition with:

- quinones
- muconic derivatives
- acid polymerization initiated by the muconic acid pendant groups from the lignin polymer

The change of the color aspect of sugarcane bagasse fiber unmodified and chemically modified is presented in Figure 1.

Unmodified   Oxidized   Modified fibers
fibers   fibers   oxidized and grafted
                              with FA
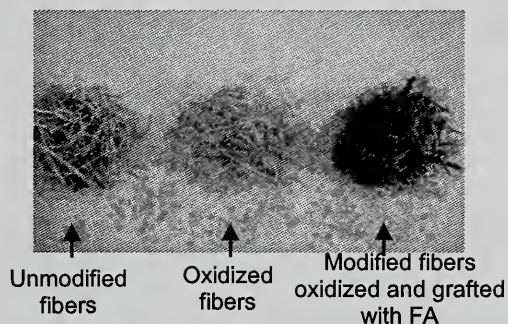
Figure 1. Sugarcane bagasse fibers.

Chemically modified fiber grafted at the surface, forming a coating of polymerized material which have a thickness about 67 μm (Figure 2).

Unmodified fiber   Modified fiber
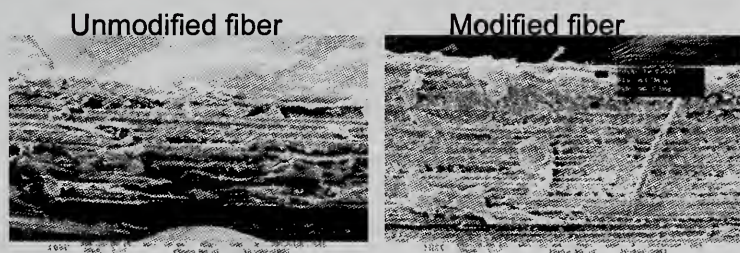
Figure 2. Cross photomicrography of sugarcane bagasse fiber (600 X).

Performance of Sugarcane Bagasse Panels

Physical Resistance

The impact strength of phenolic fiberboard (near 100 $J.m^{-1}$) was higher than those made with lignophenolic resin (near 75 $J.m^{-1}$). The difference observed was not so high and then does not represent a hindrance for the substitution of phenol by lignin.

Water Absorption Resistance

The rate of water absorption is lower for fiberboards made with unmodified fibers and lignophenolic resin than those prepared with unmodified fibers and phenolic resin. At the end, the fiberboards made with 100% lignophenolic prepolymer have better performance (Figure 3).

The chemical modification of fibers increases their affinity to the prepolymers, but also creates new microcavities at the fiber surface. The modification introduces new polar groups through oxidation (ClO_2) and reaction with FA.
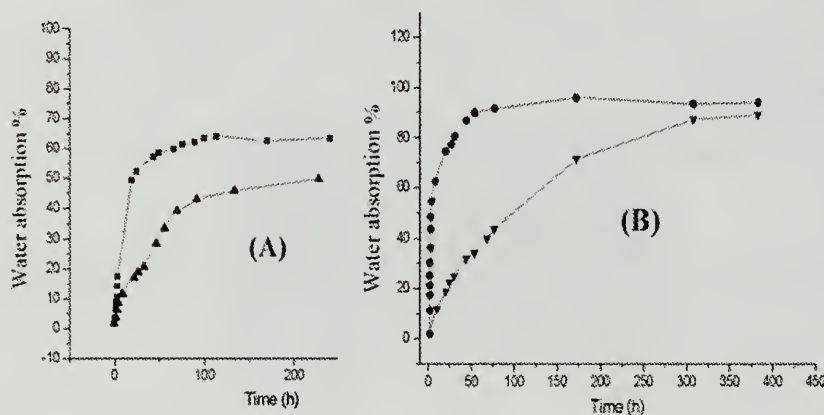
Figure 3. Rate of water absorption by panels. (A) (—▼—): unmodified fibers and phenolic resin; (—▲—): unmodified fibers and lignophenolic resin. (B) (—■—): unmodified fibers and lignophenolic resin; (—▼—): modified fibers (ClO2 + FA) and lignophenolic resin. Temperature: 25 °C.

Resistance to Fungi

All panels made with chemically modified fibers were classified as "highly resistant" when exposed to two fungi (*Trametes versicolor* and *Poria placenta, as shown in Figure 4.*
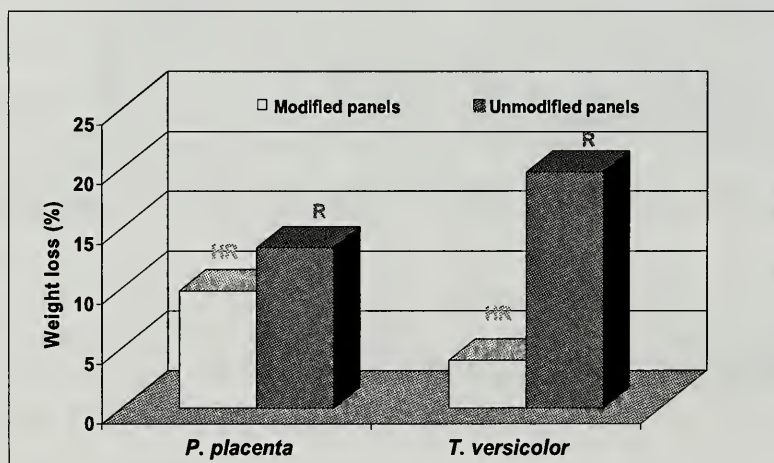
Figure 4. Weight loss of boards prepared with unmodified and modified sugaracane bagasse fibers, after an 8-week decay test, when exposed to fungi. R = resistant; HR = highly resistant.

Conclusion

A new process was developed for chemically modifying sugarcane bagasse fibers. A quite specific oxidation by chlorine dioxide of syringyl and guaiacyl phenols of the lignin polymer creating quinones or muconic derivatives was performed. The oxidized fibers were reacted with furfuryl alcohol, creating a coating around the fiber which is more compatible with phenolic resins. This modification favored the fiber-matrix interaction at the interface for the sugarcane bagasse fiberboards. To the best of our knowledge, panels entirely derived from sugarcane bagasse were prepared for the first time. They include lignophenolic prepolymer acting as resin, and chemically modified or not bagasse fibers. The chemical modification confers the panels with enhanced durability against white-rot fungi and to a less extent to brown-rot fungi. The development of an industrial process using sugarcane bagasse as a renewable raw material to prepare fiberboards for indoor and outdoor uses in tropical areas appears feasible.

Trials and Tribulations of Testing an Organic Flocculant for Colour Removal

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Introduction

The Sugar Milling Research Institute (SMRI) has long been involved with research into new and cost-effective chemicals and methods for reducing the colour of juices and syrups, and hence raw sugar, in South African sugar mills. This has been driven by several factors, principally:

- The relatively high colours of South African cane varieties. These varieties have been specifically bred for South African conditions, where drought, disease and pest resistance are paramount. As a consequence, many local varieties are thinner in diameter (with a higher proportion of coloured rind to fibre) and have relatively high colours.
- The extensive use of diffusers in the South African industry (80% of cane crushed is extracted in diffusers), and the well-known fact that higher juice colours are produced by cane diffusers than by milling tandems.
- The general improvement in sugar quality, particularly with respect to colour, in the world market.

Recently, the SMRI has been testing an organic-based flocculant, previously untested in South Africa, that is claimed by the manufacturers to deliver great benefits in terms of colour and polysaccharide reduction during mixed juice clarification. A number of laboratory trials were undertaken, with the first delivering such promising results (up to 42% colour removal with 160 ppm bioflocculant on juice) that a two-day factory trial was done at the end of the 2005/6 season at a South African factory. As a result of variable cane quality and quality requirements for the back-end refinery, the results were inconclusive.

Consequently, a number of further laboratory trials were undertaken in order to explore the possible reasons for the poor results.

Experimental

Batch clarification trials in the laboratory were planned and carried out to test the influence of a large number of factors that were considered to have an influence on the performance of the organic flocculant. These were:

- hot liming *versus* cold liming.
- flocculant addition before or after hot liming.
- flocculant addition before or after heating and at an intermediate temperature. For this and the previous point, the order of addition was considered important in terms of the chemical reactions taking place between the flocculant, the colour bodies and the lime.
- flocculant addition before or after phosphoric acid addition. The flocculant suppliers suggested that insufficient phosphate in the mixed juice might cause poor performance of the flocculant, so juice phosphate levels were measured and extra phosphate added using phosphoric acid.
- diffuser juice *versus* mill juice. Juices from diffusers and from milling tandems are known to have different mixtures of colour types, and could therefore respond differently to the flocculant, whereas the suppliers had experience with milling juices only.
- high initial *versus* low initial mixed juice colour. It was desirable to test whether the flocculant only performed well on good quality juices, or was capable of dealing with poor quality juices as well.
- double liming *versus* single liming. This was considered in terms of the chemical reactions of the flocculant.
- limed juice pH (including pH at various temperatures). The final limed juice pH is important for best clarification results, but there was some confusion about what the limed juice pH set-points were at the factories, particularly with respect to what temperature they were measured at.

Results and Discussion

Despite extensive experimental work over a range of conditions, the initial excellent results could not be replicated. This project did reveal, though, that there was generally poor understanding in the factories of the effect of temperature on pH in juices, and some factory staff were not sure at what temperature the quoted clear juice pH values were measured. It was clear that even without the use of a colour removal aid, limed juice pH did have an effect on clear juice colours (Figure 1), but care must be taken to understand the consequences on sugars of liming to a pH other than around neutral in an effort to minimise colour.
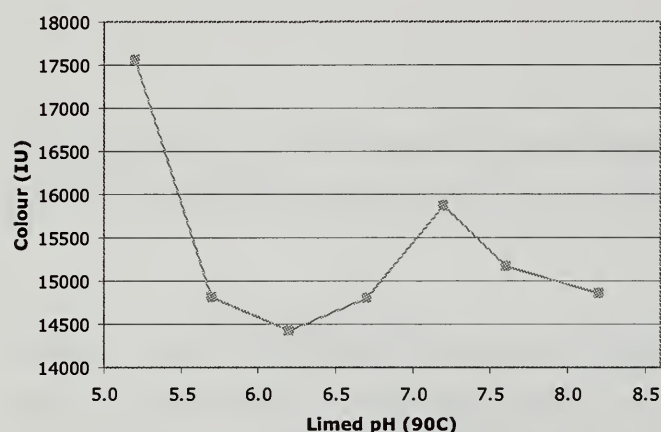
Figure 1. Effect of limed juice pH on clear juice colour

When interpreting colour removal results, it is important to consider both the percentage colour removal achieved and the actual number of colour units removed, particularly when comparing juices of widely differing initial colours. The selected results shown in Table 1 illustrate this effect. One pair of tests has the same percentage colour removal (12%), but the number of colour units removed are very different (1520 and 3380 IU), while another pair removed the same number of units (1960 and 1970 IU) but this translated to very different percentage colour removals (7% and 12%).

Table 1. Results of tests showing colour units removed and percentage colour removal

Colour removal (%)	Colour unit difference (IU)	Colour removal (%)	Colour unit difference (IU)
2	300	10	2460
3	460	12	1960
4	610	12	3380
5	1170	12	1520
5	1350	12	3380
5	730	13	1440
6	1700	13	1570
6	1750	13	2186
7	1970	14	3590
7	1060	16	2780
8	1670	19	3470
9	1460	42	7540

This project also emphasised the great variability in the properties and behaviours of different juices in clarification, even within one factory and certainly between factories and at different times of the season. To illustrate the extent of this, selected pairs of results are presented below, with the varying factors listed.

Example 1.

Juice was taken from a single factory that has both a diffuser and a milling tandem. The cane fed to each extraction line is essentially the same, but the juice colours were as follows.

Juice source	Diffuser	Mill
Control clear juice colour (IU)	27,350	11,370
Clear juice colour after treatment with 400 ppm of organic flocculant (IU)	25,380	9,930

Example 2.

The above test was repeated using juices from the same mill, but at a different time of year. Notice how much closer the diffuser and mill juices colours are.

Juice source	Diffuser	Mill
Control clear juice colour (IU)	21,440	16,310
Clear juice colour after treatment with 400 ppm of organic flocculant (IU)	19,770	15,850

Example 3.

This example shows the results of two trials using juices from another factory operating diffusers only, but at different times of year. The first trial shown was the very first run with the organic flocculant that gave such excellent results.

Trial	First	Second
Control clear juice colour (IU)	17,850	27,800
Clear juice colour after treatment with 40 ppm of organic flocculant (IU)	14,379	-
Clear juice colour after treatment with 160 ppm of organic flocculant (IU)	10,310	-
Clear juice colour after treatment with 400 ppm of organic flocculant (IU)	-	26,100

Example 4.

Here, samples of juice were tested from two different factories, both operating diffusers, but in different cane-growing areas. The large difference as a result of different varieties and cane quality is clearly evident.

Juice source	Factory A	Factory B
Control clear juice colour (IU)	23,830	14,040
Clear juice colour after treatment with 400 ppm of organic flocculant (IU)	21,370	13,740

Another point that became clear was that the use of higher dosages of the flocculant may not necessarily have been of benefit (see example 3). In terms of the nature and action of the organic flocculant, more was possibly not better, but most laboratory trials only compared the control with a dosage of 400 ppm, to reduce the number of tests and analyses that would have to be performed. This dosage was in line with the supplier's recommendation for laboratory testing, and was designed to determine the maximum benefit to be achieved. However, the best results (from the first trial) were achieved with a dosage of only 160 ppm.

The use of Indicator Value (colour measured at pH 9 divided by colour measured at pH 4) was also considered as it can give insight into the different types of colour in juices. However, this was only measured for later trials, again to reduce the number of analyses required, but did not reveal large differences between treated and untreated juices.

Conclusions

- The additional tests performed were not able to reproduce the initial good results, and the reasons for this are still not known.

- The evaluation of colour removal in a factory is highly complex and conclusions cannot be based on a single set of tests, as there are many factors to consider.

- The scale of laboratory trials is usually too small (too much scatter) while factory trial conditions cannot be sufficiently controlled.

- The issue of whether to assess results in terms of percentage colour removal or number of colour units removed is very pertinent when dealing with juices of widely differing colours.

Optimization of α-Amylase Application in the Sugarcane Industry

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Abstract

In recent years there have been warnings by some U.S. refineries that there may be a penalty for high starch concentrations in raw sugar if starch control is not improved. Most commercial α-amylases used by the U.S. sugar industry to control starch have intermediate temperature stability (up to 85° C with an optimum ~70° C), and are produced from *Bacillus subtilis*. There is neither a uniform/standard method to measure the α-amylase activity in the sugar industry nor a regulatory body to issue or regulate standard activity methods and units for any commercial enzyme. A method incorporating Phadebus[TM] blue starch tablets was modified to simulate conditions in typical last evaporators, i.e., pH 6.4 and 65.5° C, where α-amylases are mostly applied. A wide range of activity existed for α-amylases (59.0 to 545.3 KNU/ml) that did not reflect their comparative unit costs, i.e., activity per U.S. dollar only differed 4-fold from 40.7 to 161.8 KNU/ml/$. α-Amylase optimization trials in the last evaporator at three factories were conducted across the 2005 Louisiana processing season. Factory 1 typically applied 3.6 ppm/cane wt. of (undiluted) *B. subtilis* α-amylase with low activity (59 KNU/ml) for an average starch hydrolysis of 6.6% as determined with an iodometric method, that only increased to 11.4% at a 7.2 ppm dosage. Similar disappointing results occurred at Factory 3. At Factory 2, the same α-amylase (59 KNU/ml) at 10 ppm (undiluted) gave an average hydrolysis of 25.4% that only increased to 28.5% at 20 ppm. Application of a *B. subtilis* α-amylase of higher activity (545.3 KNU/ml) at 2 ppm gave an average hydrolysis of 26.7%, but only increased to 29.6% at 5 ppm because of low contact between the α-amylase and starch. Application of the α-amylase as a working solution diluted 3-fold in water at the factory improved contact and starch hydrolysis from 31.9 to 42.0% at 2 and 5 ppm, respectively, and is more cost-effective than adding it undiluted. Concern about the use of engineered high temperature stability (up to 115° C) α-amylases from *Bacillus licheniformis* and *stearothermophilus*, developed for larger markets than the sugar industry, and possible carry-over activity into raw and refined sugars, molasses, and food products, are discussed.

Introduction

Starch, formed in the sugarcane plant as a storage polysaccharide, is extracted into juice on factory milling or diffusion. Starch is present in the sugarcane stalk but is much more abundant in the leaves and growing point region. Starch is found in all sugarcane products in the factory and refinery, including raw and refined sugars. Starch concentrations in a particular product vary widely depending on country, season, variety, diurnal cycle, occurrence of sugarcane disease, sugarcane maturity, processing conditions, effectiveness of removal techniques, and method of analysis (Imrie and Tilbury, 1972). Furthermore, in recent years, starch being delivered to U.S. factories has risen markedly because of the increased production of billeted and green (unburnt) sugarcane (Godshall, *et al.*, 2000). Starch is an undesirable impurity because it causes processing difficulties in the factory and refinery (especially a carbonatation refinery). Processing costs increase not only in terms of additional processing aids but also from increased viscosity of massecuites, reduction of crystallization and centrifugation rates, occlusion of starch into the sucrose crystal, increased molasses production (Kampen, *et al.*, 1998), reduced filterability and affination of raw sugars, and impediment of refinery decolorization processes (S. Clarke, personal communication). Mud filtration is particularly impeded when a carbonatation refinery processes raw sugar containing >250 ppm/°Brix starch; moreover, the rate of throughput is dramatically slower and contractual deadlines can be missed (F. Goodrow, personal communication). For these reasons, Louisiana U.S. factories are being encouraged to deliver raw sugar containing <250 ppm/°Brix starch, with <200 ppm/°Brix preferred to a Louisiana carbonatation refinery (F. Goodrow, personal communication). In comparison, in South Africa raw sugar containing starch >130 ppm/°Brix is penalized (P. Schorn, personal communication). In the U.S., however, there is no current penalty for high starch concentrations in raw sugar. Instead, an informal policy of encouragement and cooperation exists between the carbonatation refinery and factory, which refinery staff have considered to be successful the past 3-4 years (F. Goodrow, personal communication). Cooperation includes the application of α-amylase in the factory to hydrolyze starch, and the use by factory staff of a simple colorimetric method developed by the refinery, to monitor and control starch concentrations.

Starch exists as semi-crystalline granules (1-10 μm) in sugarcane tissue and extracted sugarcane juice, and contains two glucose polysaccharides: ~19% amylose and 81% amylopectin (Vignes, 1974). Amylose is linear with the glucose molecules α-D-(1→4) linked. Amylopectin, in addition to the α-D-(1→4)-linked glucose found in amylose, also contains many α-D-(1→6)-linked branch points.

In comparison to α-amylase hydrolysis of the starch granule, hydrolysis of solubilized and gelatinized starch is much faster because starch crystallinity is lost and there is greater accessibility of the α-amylase to the exposed polysaccharides (Tester, *et al.*, 2004). Figure 1 illustrates the solubilization and gelatinization of starch in the factory. During clarification and evaporation, starch granules are heated, progressively swell, and rupture with release of amylose and amylopectin, with final transformation into an amorphous viscous solution (paste) (Figure 1). Godshall, *et al.*, (1991) have reported that some starch is solubilized leaving the last mills before clarification, especially if warm imbibition water is applied. Linear amylose molecules are capable of forming helices and can readily associate in water by hydrogen bonding to form a gel network, whereas branched amylopectin cannot (Tester, *et al.*, 2004). Amylose molecular

association phenomenon is known as retrogradation. One of the primary reasons for adding α-amylases in the factory is to stop amylose from retrograding and forming very viscous solutions after the evaporator station. α-Amylases (endo-1→4-α-D-glucan glucohydrolases; EC 3.2.1.1) are endo-hydrolases that, in the presence of water, randomly cleave 1→4-α-D-glucosidic linkages between adjacent glucose molecules in the amylose chain of the solubilized/gelatinized starch. The viscous solution is progressively "thinned" into medium and lower MW dextrins, and finally maltodextrins (oligosaccharides) of smaller chains (often in the 2-7 DP range) (Figure 2). α-Amylases are classified according to their action and properties (Pandey, *et al.*, 2000) and are derived from several bacteria, yeasts, and fungi. Most α-amylases contain a tightly bound calcium ion that is essential for having a stable, active enzyme (Vallee, *et al.*, 1959). Bacterial α-amylases, particularly from Bacillus sp., are generally preferred for commercial production and widely used in numerous industries because they have the most diverse biochemical properties (Pandey, *et al.*, 2000) and are generally recognized as safe (GRAS).

Unlike dextranases, applied in the sugar industry to hydrolyze dextran, α-amylases have high volume sales in other industries with large markets, including the baking, high fructose corn syrup, brewing, papermaking, detergent, and textile industries, and is one of the highest selling industrial enzymes (Eggleston, 2007). As a consequence, α-amylases have been subjected to many academic and industrial studies and are less expensive than dextranases (Eggleston, 2007). Industrial conditions α-amylases are applied to are frequently harsh; therefore, α-amylases have been developed, by genetic and protein engineering, to be highly active in such conditions, i.e., extreme pH's and high temperatures (Eggleston, 2007). However, as the sugar industry is a relatively small market, α-amylases have *not* been specifically tailored to its conditions.

Recent studies (Eggleston and Monge, 2005A; Eggleston et al, 2006a, b) reported that dextranase applications in the U.S. sugar industry were sub-optimal. This study was, therefore, undertaken to evaluate current α-amylase applications in U.S. sugarcane factories and, if necessary, to optimize the application and conditions.

Materials and Methods

α-Amylase Activity
The activity of α-amylases used in Louisiana factories was based on the Phadebas™ method of Novozyme (Anon, 2001), with major modifications. The method was conducted at 65° C and pH 6.4 to simulate conditions in last evaporators. Phadebas™ tablets (Pharmacia Diagnostics, U.S.), composed of cross-linked, insoluble, blue color starch polymer, were dissolved in distilled water and the starch hydrolyzed with applied α-amylase to give blue fragments. Absorbance of the resulting blue solution at 620 nm is proportional to the α-amylase activity. A standard curve was first produced based on Termamyl™ α-amylase (*Bacillus licheniformis*) of known activity (120 KNU/ml).

°Brix and Density
The mean °Brix of triplicate samples was measured using an Index Instruments TCR 15-30 temperature controlled refractometer accurate to ± 0.01 °Brix. For density, the weight in grams

of 12.5 ml of each α-amylase was measured to 4 decimal places. Results of triplicate measurements are quoted.

Starch

Starch was measured using the rapid SPRI iodometric method (Godshall, *et al.*, 2004) with modifications. Because of the large variability in the °Brix's of the different syrups collected, their dilutions in de-ionized water differed to give final °Brix values of ~15.0. For syrups entering the last evaporator approximately 37 g syrup were first dissolved in 63 ml distilled water. For syrups exiting the last evaporator, approx. 23 g syrup were dissolved in 77 ml. The syrup solution was first boiled for 5 min to completely solubilize starch. Formed blue/purple specific starch-iodine complex was measured at 600 nm. Starch was assayed in duplicate samples and concentrations are quoted as average ppm/°Brix.

General Factory Sampling

Juices before and after the addition of α-amylase in the factories were carefully stored to prevent further degradation reactions and/or microbial growth. Each sample was collected in a container (250 ml) with 5 drops of biocide (Bussan 881™, Buckman Labs., U.S.). Juices were immediately placed in ice before transportation to the laboratory, and then stored in a -20° C laboratory freezer subsequent to analyses. Before all trials, factory a-amylase applications were stopped, and the existing syrup in the evaporators flushed out for 1-2 h to equilibrate the system.

α-Amylase Trials: Factory 1

The first trial at Louisiana Factory 1 occurred on 26 Oct. 2005. Factory 1 (10,000 short tons cane/day), added α-amylase with a Pulsatron Series E metering pump, to syrup in the last (3rd) evaporator with ~18 min retention time (R_t). Syrup temperature was ~ 60° C (140° F), pH 6.3-6.4, and average °Brix of exiting syrup ~65.

α-Amylase D (59.0 KNU/ml; *Bacillus subtilis* source) was applied without prior dilution. Sampling began with 0 ppm (control) α-amylase D addition. Samples were collected just before entering the 1st body (where there was a sampling point) and exiting the last body, taking into account the estimated 36 min R_t (Eggleston and Monge, 2005b). Sampling was repeated four times, every 5 min, to constitute a sampling period. With 1 h equilibration between each sampling period, second and third sampling periods were undertaken at 3.6 and 7.2 ppm/cane wt., respectively.

α-Amylase Trials: Factory 2

Louisiana Factory 2 capacity was ~13,000 stc/day. The α-amylase was added, using a pulse feeder pump (5 GPD capacity), to the last evaporator with 18 min R_t. Syrup temperature in the last evaporator was ~65° C (150° F) and average °Brix of exiting syrup was 62-65.

The first day of sampling occurred on 4 Nov. 2005 and α-amylase D (59.0 KNU/ml) was added without any prior dilution. Sampling began with 0 ppm (control) α-amylase addition. Samples were collected just before entering and exiting the last body, respectively taking into account the estimated 18 min R_t (Eggleston and Monge, 2005b). This sampling was repeated four times, every 5 min, to constitute a sampling period. With 30 min equilibration between each sampling

period, second and third sampling periods were undertaken with 10 and 20 ppm/cane wt. α-amylase D addition, respectively.

On 8 Nov. 2005, α-amylase B (545.3 KNU/ml; *Bacillus subtilis* source), undiluted, was added at 0, 2, and 5 ppm, under the same sampling conditions as 4 Nov. 2005 described above. On 9 Nov. 2005, α-amylase B was added at 0, 2, and 5 ppm/cane wt. under the same conditions as 8 Nov., 2005, except the enzyme was applied as a working solution (diluted 3-fold [1: 2 dilution] in distilled water at the factory).

α-Amylase Trials: Factory 3

Louisiana Factory 3 capacity was ~14,000 short tons cane/day and flow rate was ~2500 gals/min. The α-amylase was added, using a Pulsatron Model LB64SA metering pump (30 GPD capacity), to the last evaporator with 18 min R_t. Syrup temperature in the last evaporator was ~62.8° C (145° F), and av.erage °Brix of exiting syrup was ~65.

The first day of sampling occurred on 21 Nov. 2005 and α-amylase B (545.3 KNU/ml; *Bacillus subtilis* source) was added. Sampling began with 0 ppm (control) α-amylase B addition. Samples were collected exiting the clarification tank (clarified juice) and the last evaporator, taking into account the estimated 52 min R_t (Eggleston and Monge, 2005b) between the clarified juice and last evaporator. This sampling was repeated five times, every 5 min, to constitute a sampling period. With 30 min equilibration between each sampling period, second, third, and fourth sampling periods were undertaken at 1, 2, and 4 ppm/cane wt., respectively, of α-amylase B addition (prepared as a 6-fold diluted working solution [1:5 dilution] in condensate water from all the evaporators).

On 22 Nov. 2005, α-amylase D (59.0 KNU/ml; *Bacillus subtilis* source) was added at 0, 4, and 8 ppm, under the same sampling conditions as 21 Nov. 2005 described above. α-Amylase D was similarly first prepared as a 6-fold diluted working solution (1:5 dilution) in mixed condensate water.

Results and Discussion

Differences in the Activities and Properties of Commercial α-Amylases Used in the Louisiana Sugarcane Industry

Similar to the problem that occurred for dextranases before 2004 (Eggleston and Monge, 2005a), a great source of confusion for factory staff about α-amylases is that the activities of commercial α-amylases cannot be compared directly because each α-amylase vendor/distributor uses a different method to measure activity. Furthermore, there is no regulatory body in the U.S. to issue or regulate standard activity methods and units for commercial enzymes (Eggleston, 2007). For example, in the U.S. units of α-amylase activity can be MWU/ml, BAU/gm and MWU/g, and activities and prices can change regularly. Consequently, there is an urgent need for a uniform method to measure α-amylase activities at the factory. A method was chosen based on commercial Phadebus[TM] tablets (contains cross-linked starch of blue color) but modified to simulate industrial conditions in factory last evaporators where most α-amylases are applied, i.e.,

65° C and pH 6.4. Although these tablets are readily available, they are slightly expensive and the method is probably too sophisticated for routine use at the factory, so we are currently developing a simpler method. Nevertheless, the modified Phadebus™ method gave accurate results and relative activities of commercial α-amylases used in Louisiana in 2005 are listed in Table 1. As for commercial dextranases applied in the U.S. sugar industry, U.S. α-amylases occurred in a wide range of activity with an approximate 9-fold difference (59.0 to 545.3 KNU/ml) that did not reflect their comparative unit costs, i.e., activity per U.S. dollar only differed 4-fold from 40.7 to 161.8 KNU/ml/$ (Table 1). In comparison, up to 20-fold differences of commercial dextranases have been measured (Eggleston, *et al.*, 2006a) that also do not reflect their comparative unit costs. The °Brix and density of the α-amylases did not reflect activities (Table 1).

Intermediate and High Temperature Stable α-Amylases

Intermediate temperature (IT) stable α-amylases are mostly produced from *Bacillus subtilus* and stable up to 85° C (Table 2). They are calcium dependent α-amylases (Table 2) but this is not a concern for sugar industry applications because lime is added during the clarification process and, therefore, free calcium concentrations are adequate. Wide variations existed in the activities of three commercial *B. subtilus* α-amylases used in the U.S. sugar industry as well as their activity per unit cost (Table 1).

Although the commercial α-amylase with the highest activity per unit U.S. dollar cost (161.8 KNU/ml/$) was High Temp. α-amylase A (Table 1) there is a serious added complication for factories that choose to apply it. This is because it is an α-amylase from *Bacillus stearothermophilus* that has been specifically engineered to have extreme high temperature (HT) stability (up to 115° C) and low calcium requirement properties (Table 2) for applications in larger markets other than the sugar industry. Thus, HT α-amylases have *not* been tailored to specific conditions in the sugar industry. HT α-amylases can be *too* temperature stable in the sugar industry, and may not denature/inactivate after application, resulting in carry-over activity into raw sugar and molasses. α-Amylase activity in the raw sugar can even carry through subsequent refinery processes and eventually reside in refined sugar, molasses, and food products, which are illustrated in Fig. 3. Moreover, two U.S. refineries have sold final molasses that contained residual α-amylase activity, to barbeque sauce manufacturers, which caused barbeque sauce to detrimentally "liquefy" (B. Loupe and F. Goodrow, personal communications). To avoid this, large customers of refineries have requested that HT stable α-amylases are *not* applied at the refinery (F. Goodrow, personal communication). Concomitantly, refineries in Louisiana have requested factories *not* to apply HT stable α-amylases (F. Goodrow, personal communication).

α-Amylases from *Bacillus licheniformis* are also referred to as HT stable, although they are usually slightly less temperature stable (up to 105° C) than those from *Bacillus stearothermophilus* (Table 2). α-Amylase from *Bacillus licheniformis* has been reported to maintain over 98% of activity after 60 min at pH 6.2 at 85° C (Morgan and Priest, 1981). It has also been reported in the Cane Sugar Handbook that Termamyl™ α-amylase from *B. licheniformis* can hydrolyze 54-81% starch in a second evaporator (~95° C) with a 1.27-6.20 g

amylase/ton cane dosage (Chen and Chou, 1993) but, unfortunately, it was not stated that it was a HT stable α-amylase.

Survey of α-Amylase Applications in Louisiana in 2005

To gain current information on α-amylase applications, a survey was sent to all Louisiana factories in 2005. Seven out of twelve factories responded and results are summarized in Tables 3 and 4. Reasons for applying α-amylases varied (Table 3). Approximately half of the factories were adhering to a request by one Louisiana carbonatation refinery (there are two refineries in Louisiana), to keep starch concentrations in raw sugars to ≤ 200 pm/°Brix. Keeping in "good working relations" with the refinery and to avoid a future starch penalty were reasonable explanations for this. In contrast, the other half of the factories applied α-amylases just to improve processing conditions, particularly the reduction of mother liquor viscosity to increase fluidity and improve crystallization (Table 3). Some of these latter factories sold their raw sugar to another Louisiana refinery that has no policy on limiting starch concentrations in raw sugar as it does not operate carbonatation clarification. Wide variations in when α-amylase was applied also existed (Table 3). Some factories applied α-amylase 24 h/day every day of the processing season to either reduce starch concentrations in raw sugar or improve processing conditions. One of these factories reported that if they stopped applying α-amylase approximately 10% longer boiling times in the vacuum pans were noted, and centrifugation operations were impacted with approximately 25% more time being required to purge molasses from crystals. In strong comparison, other factories applied α-amylase only when processing problems or high starch concentrations in raw sugar were occurring. One factory even chose not to apply α-amylase in the 2005 processing season, although α-amylase had been applied intermittently in previous seasons.

Most factories were applying IT stable α-amylases from *B. subtilus* (Table 4), with only one applying a HT α-amylase from *B. licheniformis* and another from *B. stearothermophilus* (Tables 1 and 4). Because of their IT stability, *B. subtilis* α-amylases were applied to syrup in last evaporators with temperatures ~63° C (Table 4) and estimated R_ts of 13-30 min. (Eggleston and Monge, 2005b, reported a recent theoretical calculation of ~18 min at one U.S. factory). Typically in U.S. factories there is insufficient retention time for enzyme applications. Application of α-amylase to juice at the front (milling) end of the factory is not warranted as starch still occurs as a granule and α-amylase reacts faster with solubilized starch (Godshall, *et al.*, 1991). α-Amylase cannot be applied at the clarification unit process because the very high temperatures (~96° C) and retention times (up to 1.5 h) of clarified juice would markedly reduce activity and denature the enzyme; moreover, the lime added could have an inhibitory pH effect on activity. As water is a necessary substrate in the hydrolysis reaction of α-amylase (Figure 2), the application of α-amylases after the evaporator station is also unwarranted because of low water activity/high °Brix in massecuites. The optimum pH range for *B. subtilis* α-amylase activity is pH 5.3-8.5 with the lower end of the range more preferable (Table 2), and this generally coincides with pH conditions in last evaporators (pH 6.3-6.5).

Most factories applied α-amylase directly from the tote (barrel) without pre-mixing in condensate water or any type of water (Table 4). Many factories did not measure starch

hydrolysis across the evaporators, although a few monitored starch concentrations in the raw sugar and adjusted α-amylase applications accordingly (Table 4). For those factories that did measure starch hydrolysis there was no uniform method to measure starch, although most were based on a spectrophotometric assay (Table 4).

Factory Trials on α-Amylase Applications in Syrups

Due to the present concern of carry-over activity from HT α-amylases produced by *B. licheniformis* and *stearothermophilus*, α-amylase factory trials in 2005 were only conducted with IT *B. subtilis* α-amylases. However, the two commercial *B. subtilis* α-amylases applied in the trials differed widely in activities: 59.0 - 545.5 KNU/ml (Table 1). α-Amylases were applied to factory last evaporators to ensure the starch was solubilized and gelatinized, and to maximize the highest available retention times with the lowest temperatures in the evaporator station.

Factory 1 α-Amylase Trial

Staff at Factory 1 had been intermittently applying low activity α-amylase D (59 KNU/ml; *B. subtilis*) to their last evaporator syrup when starch concentrations in raw sugar were >200 ppm/°Brix. However, they were uncertain if starch hydrolysis was occurring. As a consequence, a factory trial was undertaken, applying α-amylase D under the same conditions as the factory, i.e., at 3.6 ppm with no prior dilution before application, as well as at 7.2 ppm. As seen in Figure 4, starch concentrations in the syrup were relatively high at 11,850 ppm/°Brix because it was early season when the sugarcane was immature. A disappointing average 6.6% hydrolysis of starch was measured at 3.6 ppm dosage that increased only to 11.4% at 7.2 ppm. The iodometric method (Godshall, *et al.*, 2004) to measure starch concentrations most likely underestimated some starch hydrolysis because a negative starch value requires starch at ~DP 18 or lower (Bailey and Whelan, 1961). Furthermore, for complete hydrolysis of starch to glucose, glucoamylase must be added to hydrolyze 1→6 glycosidic bonds in the amylopectin molecule, although there are much less of these bonds compared to 1→4 bonds that α-amylase hydrolyzes. Nevertheless, although starch hydrolysis results in this paper may be underestimations, the relative results are valid for comparisons. Factory 1 hydrolysis results (Figure 4) were still relatively low and, moreover, when the factory stopped α-amylase application they did not notice any differences in the efficiency of the subsequent boiling or centrifugation processes.

As a result of this trial, the factory staff decided it was not economical for them to add α-amylase D of low activity (Table 1).

Factory 2 α-Amylase Trial

For the first trial at Factory 2 the same low activity α-amylase D (59 KNU/ml; *B. subtilis*) investigated at Factory 1 (Figure 4) was applied. As a 7.2 ppm dosage at Factory 1 gave only 11.2% av. starch hydrolysis, in this trial dosages of 10-20 ppm of undiluted α-amylase D were investigated. Although Factory 2 syrup had slightly lower starch concentrations (~1600 ppm/°Brix) than Factory 1 (compare Figure 5 with Figure 4), average starch hydrolysis at 10 ppm was better at 25.4%. However, doubling the dosage to 20 ppm only increased the average starch hydrolysis to 28.5%, which is uneconomical (Table 5). This is most likely attributable to

low contact between the substrate and enzyme. Smaller volumes of higher activity enzyme will take longer to disperse in the juice and have less contact with the substrate (Eggleston, *et al.*, 2006a). This problem has been reported for "concentrated" dextranases in the sugar industry (Eggleston, *et al.*, 2006a) and solved by applying a working solution of the enzyme.

For the second trial at Factory 2, another *B. subtilis* commercial α-amylase B (545.3 KNU/ml), approximately 9X more active than α-amylase D, was applied undiluted at 2 and 5 ppm. At 2 ppm dosage there was an average starch hydrolysis of 26.7% that increased only slightly to 29.6% at 5 ppm (Fig. 6). Economic comparisons of the factory application of *B. subtilis* α-amylases B and D are listed in Table 5. For every U.S. dollar spent applying the high activity *B. subtilis* α-amylase B, US$4.20 had to be spent on the low activity *B. subtilis* α-amylase D for an equivalent starch hydrolysis (Table 5). This is mostly attributed to the relative activities of the enzymes not reflecting their relative unit costs (Table 1).

As there was inadequate contact between the starch and α-amylase (Figures 4 and 5), a third trial was conducted at Factory 2 applying α-amylase B (545.3 KNU/ml) as a working solution, i.e., pre-diluting α-amylase B 3-fold (1:2) with distilled water at the factory. There was a marked improvement in starch hydrolysis over the undiluted α-amylase B at the same 2 and 5 ppm dosages (compare Figure 7 with Figure 6). Average hydrolysis at 2 ppm was 31.9% (compare 26.7% when the same α-amylase was added undiluted; Figure 6). At 5 ppm the average hydrolysis was 42.0% (compare only 29.6% when the same α-amylase was added undiluted; Figure 6). This strongly indicates that preparing a working solution of α-amylase at the factory improves contact between the starch and α-amylase and increases the hydrolysis of starch. The extra water would also have aided the hydrolysis of starch because water is a necessary reactant. More importantly, adding a working solution of the high activity α-amylase is less expensive than adding an undiluted low activity α-amylase. However, factory staff should be alerted to the storage needs of such working solutions of α-amylase. As for dextranase working solutions (Eggleston, *et al.*, 2006a), they most likely begin to lose their activity after 24-48 h. It is, therefore, strongly recommended that only enough working solution is prepared for usage over a conservative 12 h period.

Factory 3 α-Amylase Trial

Factory 3 had the largest capacity (14,000 stc/d capacity) of the three factories where the trials were conducted. For the first trial at Factory 3 commercial α-amylase D (low activity 59 KNU/ml; *B. subtilis*) was applied at 4 and 8 ppm. The α-amylase was first diluted 6-fold in condensate water from the evaporators. As seen in Figure 8, starch concentrations at Factory 3 were markedly lower (~800 ppm/°Brix) than at Factories 1 and 2 (Figsure 3-5) because the trial occurred later in the season (end of November) when the sugarcane was mature. At 4 ppm, average starch hydrolysis was 9.3%, and doubling the dosage to 8 ppm only increased hydrolysis to 13.4% (Figure 8). These results are very similar to the low starch hydrolysis at Factory 1 applying the same enzyme (compare Figure 8 with Figure 4). The slight differences can be attributed to the different factory conditions and starch concentrations, adding a working solution compared to an undiluted enzyme, and slightly varying dosages. Nevertheless, these results further suggest that low activity α-amylase does not work efficiently under sugar factory

conditions, even when added as a working solution. The addition of a higher activity α-amylase is unequivocally more efficient and economical (Table 5).

Another trial was conducted at Factory 3 using the high activity *B. subtilis* α-amylase B (545.3 KNU/ml), first diluted 6-fold in condensate water from the evaporators. At only 1 ppm dosage an average 21.4% starch hydrolysis was measured. However, this only increased to 24.6% at 2 ppm dosage, and decreased to 22.9% at 4 ppm, although this was insignificant at the 5% probability level. At this current time the authors do not have a reasonable explanation for this and intend to investigate it further.

Conclusions

Like for dextranases applied in the U.S. sugar industry, a wide range of activity also existed for α-amylases. An approximate 9-fold difference existed (59.0 to 545.3 KNU/ml), that did not reflect their comparative unit costs, i.e., activity per U.S. dollar only differed 4-fold from 40.7 to 161.8 KNU/ml/$.

Optimization trials for IT stable α-amylases applied to syrup in the last evaporators of three sugarcane factories were conducted in the 2005 Louisiana processing season. The application of *B. subtilis* α-amylase with low activity (59 KNU/ml) gave disappointing and uneconomical starch hydrolysis results at two factories. In comparison, the application of a higher activity (545.3 KNU/ml) *B. subtilis* α-amylase gave more economical results, although problems existed because of low contact between enzyme (α-amylase) and substrate (starch) and the low water activity of syrup. However, application of the high activity *B. subtilis* α-amylase as a working solution diluted 3-fold in water at the factory, significantly improved starch hydrolysis (up to 42%) and was more cost-effective than adding the α-amylase undiluted. Further starch hydrolysis across the syrup tanks after the last evaporators was also studied (results not shown) with none detected which may reflect even lower water activity. Application of IT α-amylase in the penultimate evaporator may provide more water reactant, although the syrup temperature is higher (104° C) than the last evaporator.

Variations in sugarcane starch concentrations across the season and, to a lesser extent, processing variations make it difficult to have a standardized addition of α–amylase in raw sugar manufacturing. Starch hydrolysis may be improved by the co-application of glucoamylase (exo-1→4-α-D-glucan glucanohydrolase; EC 3.2.1.3) that hydrolyzes single glucose units from the non-reducing ends of amylose and amylopectin in a stepwise manner. Moreover, it can hydrolyze the 1→6-α-linkages at the branching points of amylopectin (although at a lower rate than 1→4 linkages) that is mutually compatible with α-amylases (Pandey, *et al.*, 2000).

Concern about the use of recently engineered HT stable (up to 115° C) α-amylases from *Bacillus licheniformis* and *stearothermophilus*, developed for much larger markets than the sugar industry, and possible carry-over activity in raw and refined sugars, molasses, and food products, was discussed. Currently large customers of refinery products request that HT α-amylases are not applied in the refinery, and refineries request they are not applied in the factory. In other industries, a major advantage of HT α-amylases is the reduction in required retention time

(Pandey, *et al*., 2000).   Researchers at the Audubon Sugar Institute (Anon, 2003) conducted a pilot plant study with HT α-amylase applied to clarified juice. Although at a 5 ppm dose, starch hydrolysis was nearly complete within 5 min, about 30% carry-over activity was measured in raw sugar (Anon, 2003). It was stated that the application of a lower dose of 1 ppm may be sufficient for complete hydrolysis after 1 h. However, B. Montes (personal communication) applied a HT α-amylase (323.5 KNU/ml) to the clarification tank at a Louisiana factory in 2005 and found no starch hydrolysis occurred because of enzyme denaturation at such high temperatures and long retention times (~96° C and 1.5 h). Furthermore, residual activity after 1 ppm dose may still occur in the raw sugar, and factory studies are needed to ascertain this.

In the short-term, as the sugar industry is a small α-amylase market, limited or no research and development is expected by large enzyme companies to tailor α-amylases' properties to the harsh factory processing conditions, e.g., better performance at the high °Brix/low water conditions in last evaporator syrups. Consequently, optimization studies to improve the operating conditions of α-amylases as outlined in this paper are the best solutions for the near future. The use of uniform ultrasound technology (Yachmenev and Lambert, 2007) to improve contact between the α-amylase and starch will only enhance industrial optimization.

More long term solutions to overcome the sugar processing constraints of α-amylases could be protein engineering of the enzymes using, for example, site-directed and random mutagenesis techniques (Eggleston, 2007) to tailor the properties of α-amylases to sugar industry conditions.

Recommendations

1. Measure the activity of the α-amylases at the factory to i) economically compare α-amylases, ii) monitor activity changes on storage, and iii) check the activity of different batches delivered.
2. Request to know the *Bacillus* source of the α-amylase to determine if it is intermediate or high temperature stable.
3. Prepare a working solution of the α-amylase (preferably of relatively high activity) with water at the factory to improve starch hydrolysis in the last evaporators.
4. Until further research has been accomplished to ascertain the carry-over activity of low dosage applications of HT stable α-amylases, only apply intermediate stable α-amylases at the factory.

Acknowledgements

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Table 1. Sources and relative activities of α-amylases used in Louisiana raw sugar manufacture in 2005.

Commercial α-amylase[a]	Microorganism Source	Color	°Brix	Density	Activity KNU/ml at 65 °C and pH 6.4	Activity/ U.S. $
A	*Bacillus subtilis*	Dark brown	23.8	1.10	120.4	82.5
High Temp. A[b]	*Bacillus stearothermophilus*	V. dark brown	38.3	1.20	323.5	161.8
B	*Bacillus subtilis*	V. dark brown	32.1	1.12	545.3	118.3
C	*Bacillus licheniformis*	Dark brown	26.6	1.05	73.5	58.8
D	*Bacillus subtilis*	Dark brown	23.8	1.06	59.0	40.7

[a] The prices of the different α-amylases ranged from $1.25 to $4.61 per lb in 2005

[b] High Temp A α-amylase and α-amylase A were sold by the same company

Table 2. Properties of α-amylases from different *Bacillus* sources used in the Louisiana sugar industry.

α-Amylase Source	Optimum Temp. °C	Maximum Effective Temp. °C	Optimum pH Range	Effective pH range	Calcium Requirement for Thermal Stability ppm	References
Bacillus subtilis	70 °C (158 °F)	Up to 85 °C (185 °F)	5.5 - 8.5	5.0 - 10.0	150-400	Haki and Rakshit, 2003
Bacillus licheniformis	90 °C (194 °F)	Up to 105 °C[a] (212 °F)	5.5 – 9.0	5.0 - 9.0	5-40	Haki and Rakshit, 2003; Nielsen and Borchert, 2000; Ivanova et al, 1993
Bacillus stearothermophilus	95 °C[b] (203 °F)	Up to 115 °C (239 °F)	5.5 - 6.5	4.0 - 7.0	0[c]-75	Hashida and Bisgaard-Frantzen, 2000

[a] 60% activity at 100 °C (212 °F)
[b] At pH 6.0
[c] Termamyl LC[TM] (commercial α-amylase from Novozymes) requires no calcium ions (Hashida and Bisgaard-Frantzen, 2000)

Table 3. Results from survey of α-amylase applications in Louisiana in 2005. Reasons for adding α-amylase.

Factory	α-Amylase used[a]	Reason(s) for Adding α-Amylase	When Do They Add α-Amylase?
1.	D A C	To reduce viscosity and improve crystallization	Amylase is added 24 h/day for every day of the processing season
2.	D	To increase fluidity of mother liquor	Only when processing problems are occurring
3.	A	To avoid problems of high viscosity and to have good raw sugar quality.	Only when high starch levels are occurring in raw sugar
4.	A	Adding amylase under the assumption that starch >200 ppm/°Brix in raw sugar the refinery will complain. Also they believe that some benefit is gained in the boiling house.	Amylase is added 24 h/day for most days of the processing season
5.	A	To meet refinery starch levels of 200 ppm/°Brix raw sugar and as a consequence keep in good working relations with the refinery	Amylase is added 24 h/day for most days of the processing season
6.	High Temp A	To keep starch limits low in the raw sugar and gain benefits in the boiling house and centrifugation station	Amylase is added 24 h/day for most days of the processing season
7.	None	To keep starch levels low in the raw sugar at the refinery's request	Only when high starch levels are occurring in raw sugar

[a] See Table 1

Table 4. Results from survey of α-amylase applications in Louisiana in 2005.

Factory	α-Amylase used[a]	Point of application	Dosage	Temp °C (°F)	Estimated R_t	pH	How Applied	How test starch hydrolysis	Storage
1.	D A C	Last Evaporator	2-4ppm/ cane	63-68 (145-155)	20min +10min in syrup tank	6.2-6.5	Amylase mixed 1:5 with condensate water	Modified ASI/SPRI method	In a room at ±15 to 27°C
2.	D	Last Evaporator	3.6 ppm	55 (130)	18 min	6.3	Amylase mixed 1:5 with water then pumped	None	Room temperature
3.	A	Syrup	2-5g/ton cane	60 (140)	30 min	6.3	No extra mixing	No test	2-3 weeks Storage time
4.	A	Last evaporator	2.3g/ton cane	~60 (140)	13min	6.0-6.4	Directly from tote; no extra mixing or dilution	KI colorimetric method; only raw sugar	Stored in yard (seldom have > 1-2 totes on site)
5.	A	Syrup	.001gals /ton	46 (115)	--	6.5-6.8	No additional mixing	Simplified modified method; only raw sugar	Room temp in warehouse
6.	High Temp A	First to last evaporator	1-8ppm as needed	~63 (145)	18 min	6.2-6.5	No additional mixing	SPRI method	Air conditioned room
7.	None[b]	--	--	--	--	--	--	Refinery spec method	--

[a] See Table 1 [b]Did not add amylase in 2005 processing season because except for two days at the start of the season the starch levels in the raw sugar were not high enough, but have added it previously

Table 5. Cost-effective calculations for different *B. Subtilus* α-amylase applications at Factories 1 and 2. α-Amylases B and D were undiluted before application.

Commercial α-amylase/activity	Factory[a]	Dosage (ppm)	Starch hydrolysis (%)	% Starch hydrolysis/ppm dosage (%/ppm)	Relative equivalent cost (U.S. $)
α-amylase B	1	3.6	6.6	1.83	
(545.3 KNU/ml)[b]	1	7.2	11.4	1.58	
	2	10	25.4	2.54	1[c]
	2	20	28.5	1.43	
α-amylase D[b]	2	2	26.7	13.35	4.2[c]
(59.0 KNU/ml)	2	5	29.7	5.94	

[a] For factory and experimental conditions see MATERIALS AND METHODS section
[b] See Table 1
[c] The best % starch hydrolysis/ppm dosage for each α-amylase were taken
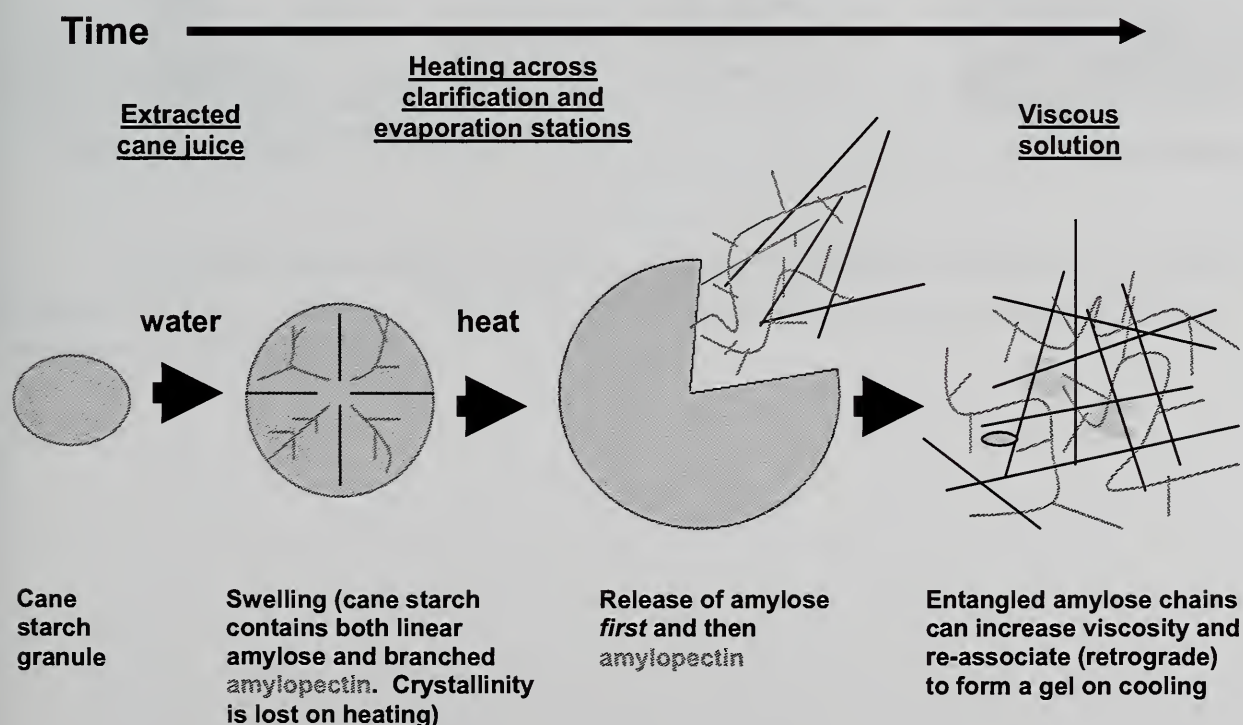
Figure 1. Starch solubilization and gelatinization across the sugarcane factory.
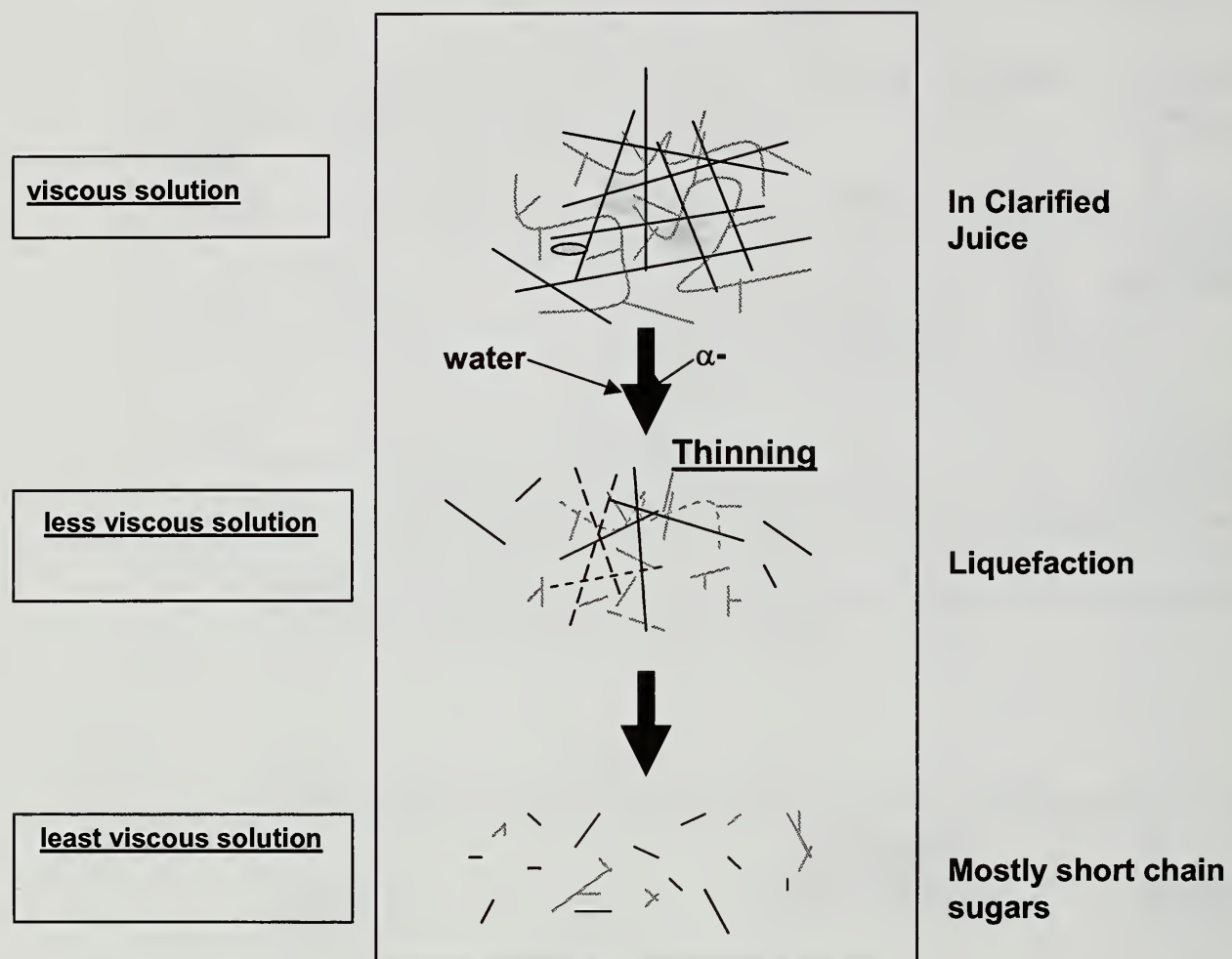
Figure 2. Action of α-amylase on gelatinized, viscous solution of starch in the sugarcane factory.

Raw sugar

Molasses

Refined

Food samples
are
detrimentally "liquefied"
by residual HT α-amylase activity
in molasses or refined sugar

Barbeque sauce

Starch pudding
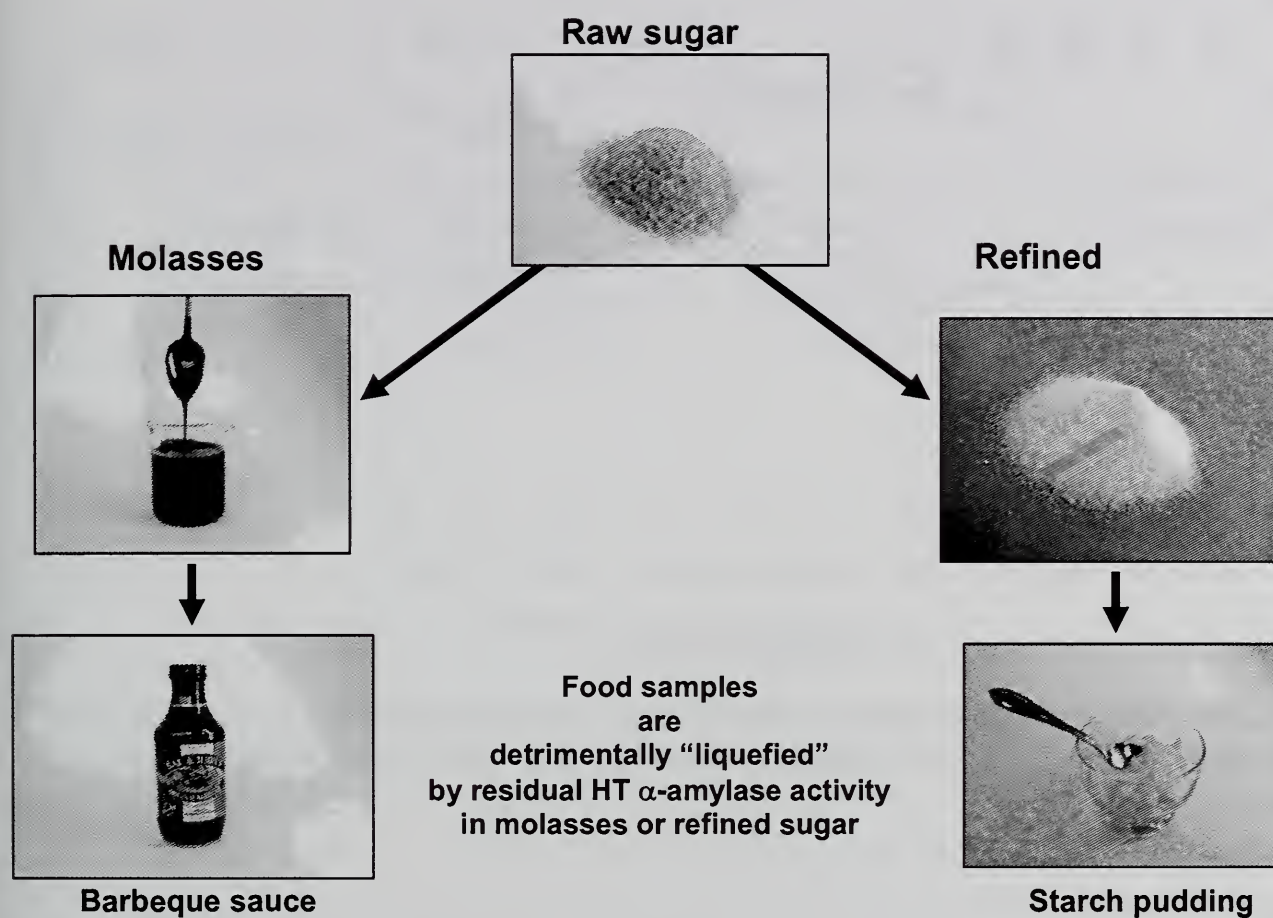
Figure 3. Known carry-over activity of high temperature stable α-amylases into sugar, molasses, and food products.
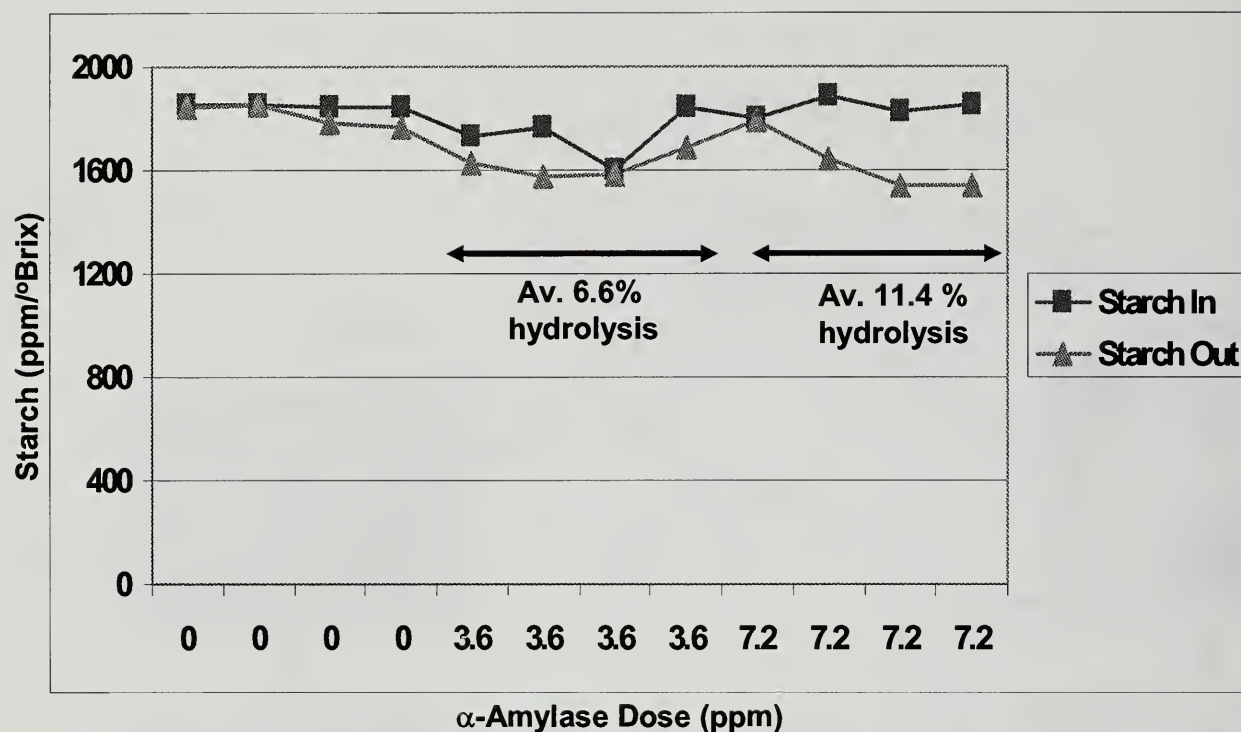
Figure 4. Effect of applying α-amylase D from *Bacillus subtilus* (59 KNU/ml activity) at Factory 1 on 26 Oct, 2005. Factory conditions: last evaporator; 3.6-7.2 ppm undiluted enzyme; R_t=18 min; ~60° C.
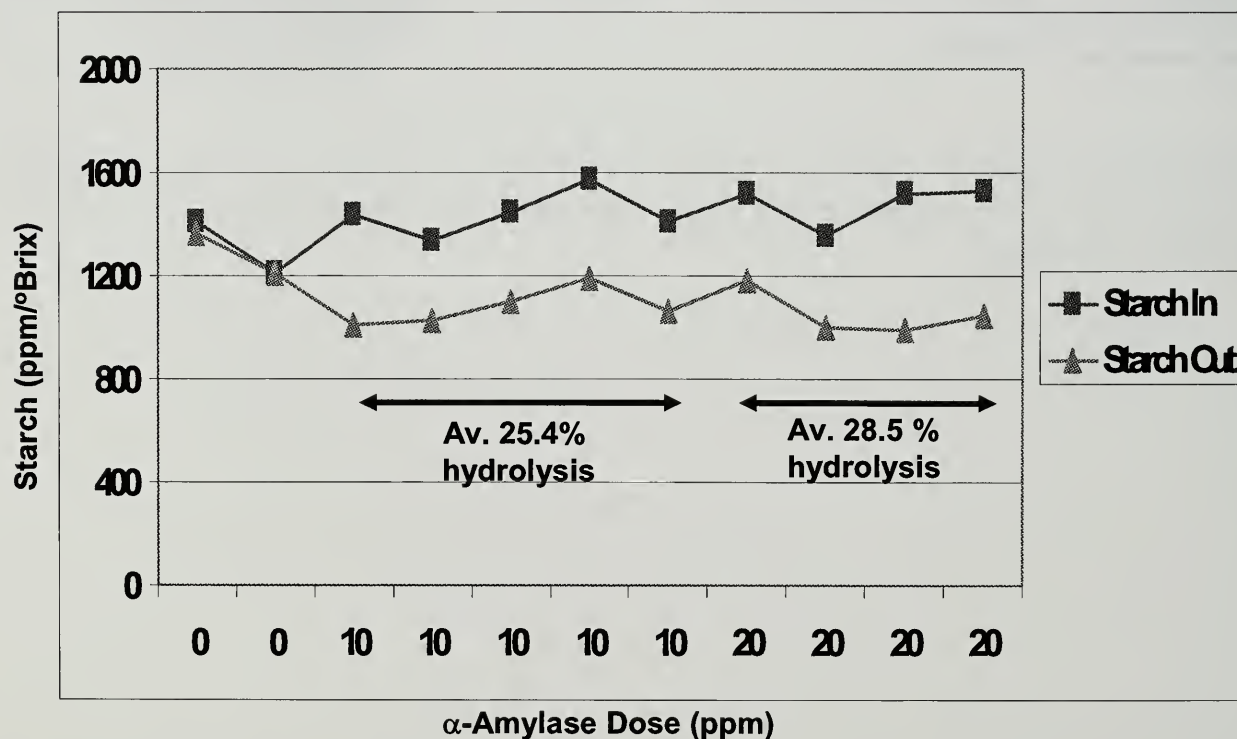
Figure 5. Effect of applying α-amylase D from *Bacillus subtilus* (59 KNU/ml activity) at Factory 2 on 4 Nov, 2005. Factory conditions: last evaporator; 10-20 ppm undiluted enzyme; R_t=18 min; ~65° C.
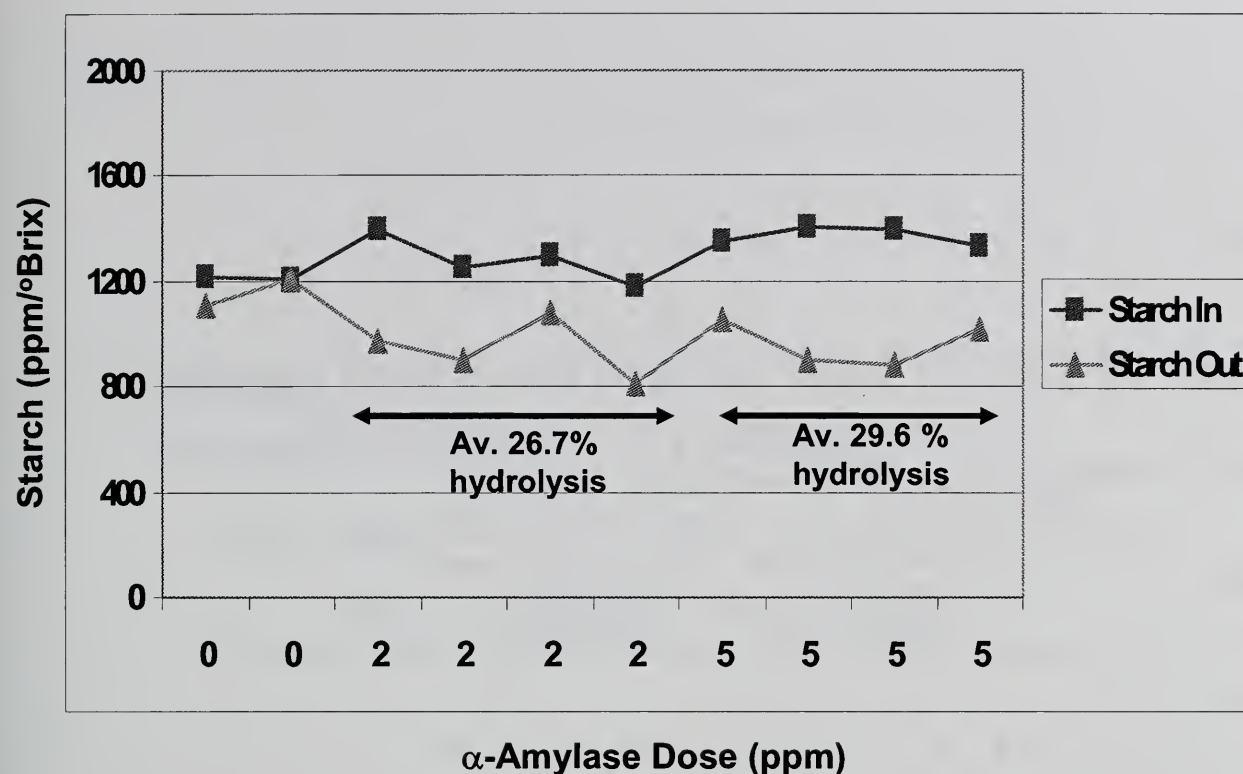
Figure 6. Effect of applying α-amylase B from *Bacillus subtilus* (545.3 KNU/ml activity) at Factory 2 on 8 Nov, 2005. Factory conditions: last evaporator; 2-5 ppm undiluted enzyme; R_t=18 min; ~65° C.
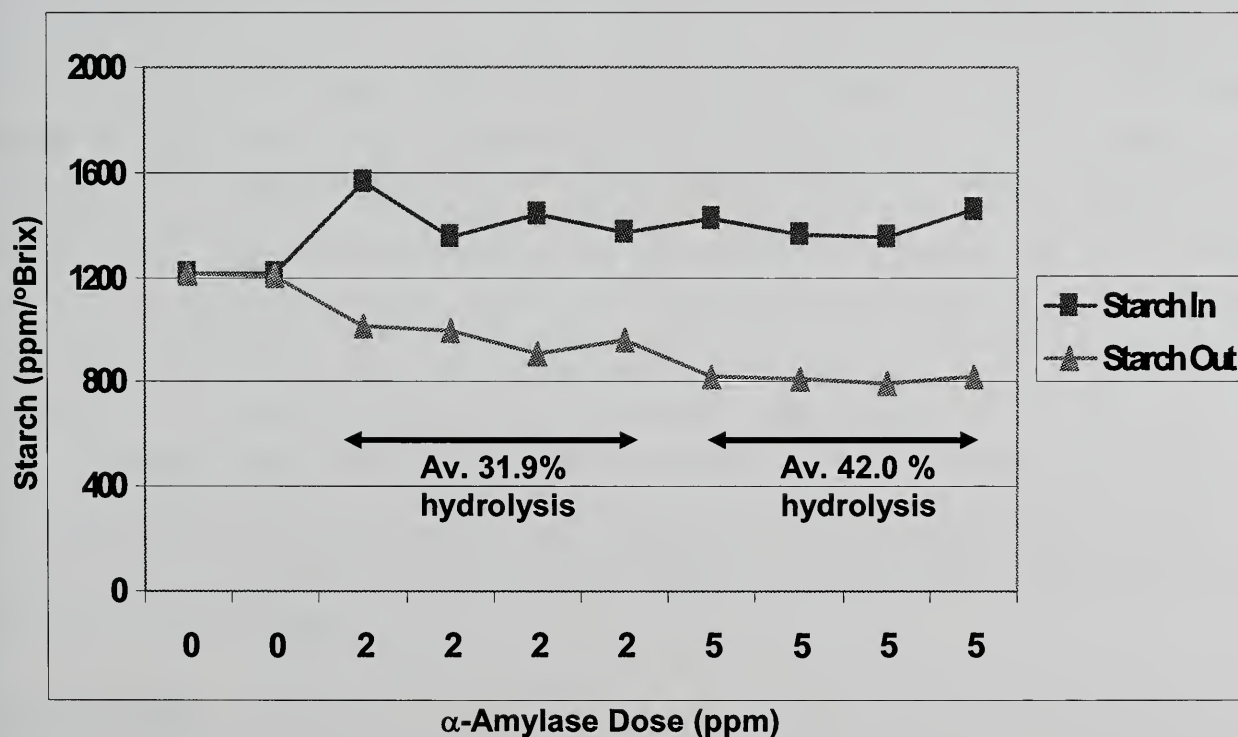
Figure 7. Effect of applying α-amylase B from *Bacillus subtilus* (545.3 KNU/ml activity) at Factory 2 on 9 Nov, 2005. Factory conditions: last evaporator; 2-5 ppm 3-fold diluted working solution; R_t=18 min; ~65° C,
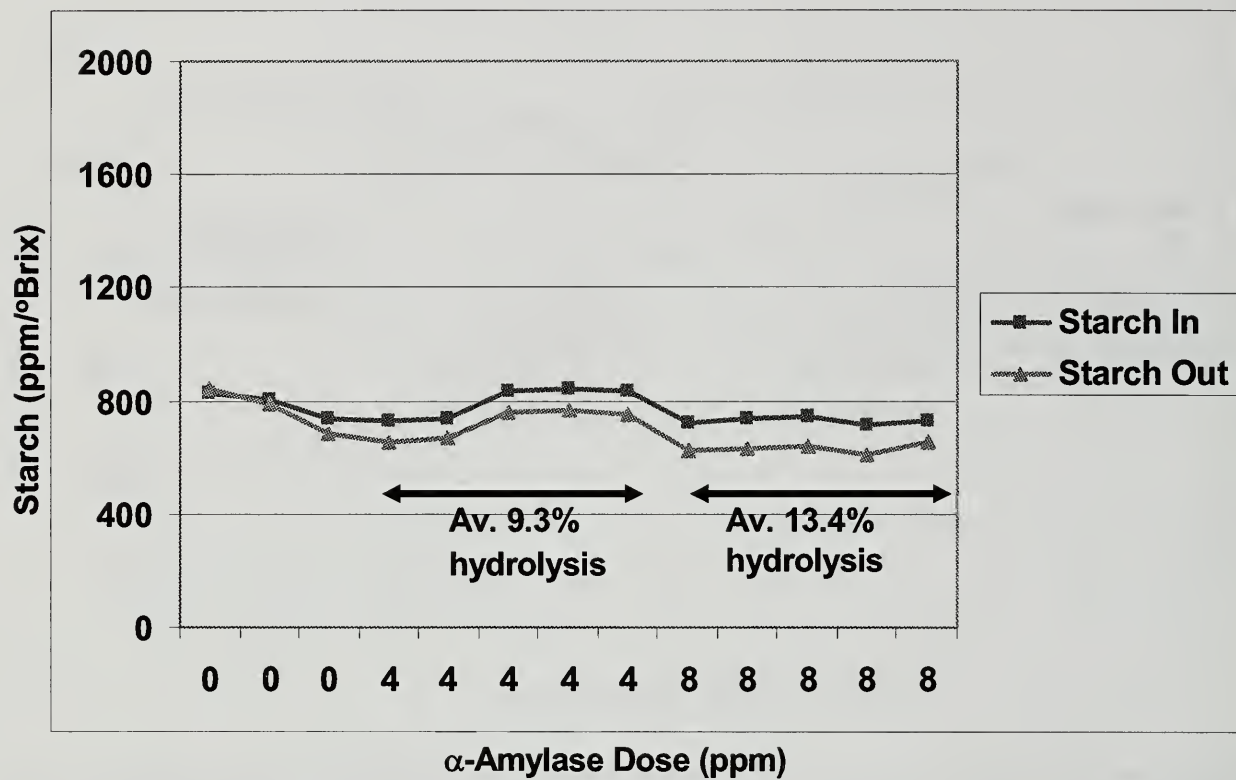
Figure 8. Effect of applying α-amylase D from *Bacillus subtilus* (59 KNU/ml activity) at Factory 3 on 21 Nov, 2005. Factory conditions: last evaporator; 4-8ppm 6-fold diluted working solution; R_t =18 min; ~63° C.

Sugarcane Green and Brown, Dried Trash Effects on Processing: A Preliminary Study

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Abstract

Currently, there is a dramatic shift world-wide from the harvesting of burned to unburned (green) sugarcane. With increased pressure from public and environmental agencies to further restrict or curtail burning in the U.S. and many other countries, even more unburned sugarcane with extra impurities (trash, i.e., leaves and tops) are expected to be delivered to factories, putting added burdens on processors to deal with and/or remove them during processing. The effect of changing to "green" harvesting on processing has not been properly or fully characterized and, therefore, very few solutions to minimize the detrimental processing effects of trash have been developed or implemented. The effect of different percentages (up to 25% by fresh weight) of green and brown, dried trash on juice quality of commercial sugarcane var. LCP 85-384 are reported in this preliminary study. The pH and °Brix of the juices did not significantly change with increasing amounts of green trash, but increased when 25% brown, dried trash was added. The differences in °Brix indicate that brown, dried trash contributes more than green trash to the factory delivery of soluble solid impurities that have a negative impact on processing. Fiber amounts increased when both types of trash were added, but significantly more with brown, dried trash. Brown, dried trash can still be metabolically active, as indicated by the presence of starch. In general, unscreened clarified juice from juices containing brown, dried trash had more and larger (up to ~220 μm) microparticles than green trash clarified juices. Thermal processing properties are also discussed.

Introduction

In recent years in the U.S., there has been a dramatic shift from harvesting whole-stalk to billeted cane, and from burned to green (unburned) sugarcane. With green cane harvesting there has been a concomitant increase in impurities experienced by sugarcane processors that have made

processing more difficult. Such impurities occur because of associated plant extraneous matter or trash, i.e., leaves and plant tops. Knowledge of how the concentrations and physico-chemical properties of the different trash impurities from sugarcane produced from these new harvest systems impact the processing quality of juice, syrups and other sugar products, will underpin efforts to develop tests/methods to predict associated processing problems and allow for improved efficiency of affected unit processes in the factory.

Not enough is known about the amount and impact of extra impurities entering the factory with green sugarcane. What is currently known is that trash increases the amount of fiber entering and passing through the factory (Bernhardt, *et al.*, 2000) and recoverable sugar (Mrini and Fares, 2005), and the leaves and tops contain more non-sucrose impurities such as high inorganic ash (Irvine, 1978) and color (Scott, *et al.*, 1978). Kestose oligosaccharides that can deform the crystal shape (Morel Du Boil, 1991) are more abundant in leaves and the growing point region of the cane plant (Eggleston and Grisham, 2003). Concentrations of starch are also higher in tops and leaves, although concentrations change dramatically throughout the diurnal cycle, and other polysaccharides are most likely present.

Very little has been reported on the different effects that brown, dried leaves have on processing. Brown, dried leaves consist of ~5% fresh weight of the harvested cane plant, green leaves ~6%, and green tops ~12%, although these vary with commercial cane variety, if ripener has been added, and time in season (Eggleston, *et al.*, 2007). Brown dried leaves generally contain ~76-87% dry solids and green leaves ~30-43% dry solids (Arceneaux and Davidson, 1973). Brown, dried trash has been observed to cause more loss of sucrose to bagasse (Arceneaux and Davidson, 1973).

Materials and Methods

Field Experiments

Sugarcane (commercial variety LCP 85-384) plant cane was grown and combine harvested with extractor fans off on 14 December, 2005, at the Magnolia Plantation, Schriever, Louisiana. Planting occurred in September 2004 on commerce silt loam soil; experimental design was a randomized complete block with three replications. Plots were cultivated and fertilized according to recommended practices; pesticides were applied as required. Billeted stalks and trash were randomly removed from the plot and transported to the nearby Ardoyne Farm of the USDA-ARS-SRRC Sugarcane Research Unit at Shriever, Louisiana. The green (GT) and brown, dried (BT) trash were removed by hand from billets and placed into separate piles. The billets and associated trash fresh weight percentages (3 replicates each) were separately passed through a pre-breaker (Cameco, U.S.). A shredded sample (1000 g) was then passed through a core press (Cameco, U.S.) to extract juice, and produce filter cake. A sub-sample of the juice (25 ml) was heated to 95°C on a Multi-Blok™ heater (Lab-Line, U.S.) to denature natural amylase enzymes in the juice; this sample was then frozen and stored until analyzed for starch. The remainder of the juice was immediately analyzed for pH, then treated with biocide (Bussan 881, Buckman Labs., U.S.), frozen, and subsequently transported to the temporary analytical laboratory of Dr. Eggleston in Baton Rouge, Louisiana.

Fiber
The pressed filter cake from the core press was weighed, dried in an industrial TPS Lunaire oven at 65.5°C for 72 h, and reweighed. Percentage fiber was calculated as the % difference between the wet and dry weights.

pH
The pH of the juice was measured immediately after extraction, and before biocide was added, on a Model SA 720 Orion pH meter at room temperature (~25°C).

Brix
The mean Brix of triplicate samples was measured using an Index Instruments TCR 15-30 temperature controlled refractometer accurate to ± 0.01 Brix.

Color
Color was measured at 420 nm and calculated according to the official ICUMSA method GS2/3-9 (1994). Samples were prepared with triethanolamine/HCL buffer at pH 7 and filtered through 0.45 μm filters.

Starch
Starch was calculated using the rapid SPRI iodometric method (Godshall, *et al.*, 2004) with modifications. Juice was boiled for 10 min to completely solubilize the starch granules in the juice. The formed blue/purple specific starch-iodine complex was measured at 600 nm. Starch was assayed in duplicate samples and concentrations are quoted as average ppm/Brix.

Laboratory Clarification
Juice (1 L) was heated over a hot plate with constant stirring to 92°C, milk of lime (MOL) (10 Baumé) was added with stirring until the juice pH reached 7.4. Flocculant (Stockhausen polyanionic) solution (0.1%) was then added at 5 ppm using a micropipette. The limed, flocculated, heated juice was rapidly heated to boiling with constant stirring for 1 min to remove interfering bubbles, and then poured into a settling tube (5 x 34 cm) in a glass water bath (96°C) to a volume of 400 ml and stoppered immediately. After 30 min settling, a digital photograph of the cooled sample of decanted clarified juice was taken with an Olympus MIC-D. The mud settled at the bottom of the settling tube, was also observed under a digital microscope.

Thermal Properties of Juices
The thermal conductivity ($Wm^{-1}C^{-1}$) and resistivity (cMW^{-1}) were measured using a KD2 Thermal Properties Analyzer (Decagon, U.S.). The needle probe was held by a clamp to minimize vibrations and the needle inserted into the middle of a beaker (100 mL) of juice or syrup. Results are expressed as an average of six measurements.

Viscosity
Juice viscosity was measured on a Brookfield (Middleboro, US) DV-II+ rotational viscometer at 25°C. Because of the low viscosity of the juice samples, a special low viscosity ULA™ adaptor was used, to ensure torque was >10%. Temperature was maintained via a jacketed adaptor sample cell (16 mL) that was connected to a Neslab RTE-100™ water bath accurate to ±0.1°C.

The shear rate applied was 150 rpm. Viscosity in centipoises (cP) was calculated as % torque x the spindle factor.

Statistics
Means comparisons were undertaken using PC-SAS 9.1.2 (SAS Institute, Cary, NC) using Duncan's New Multiple Range Test following one-way ANOVA.

Results and Discussion

Brix (% Dissolved Solids) and Fiber
The effect of varying amounts of trash on the Brix (% dissolved solids) and fiber content of juices with varying amounts of added green or brown, dried trash are illustrated in Figure 1.

As expected, fiber amounts increased when both GT and BT were added, but significantly more with BT. Brown, dried trash contributes more to increased fiber and dissolved impurity solids than green trash. Such impurity solids may consist of degraded and intact polysaccharides.

Viscosity
The effect of varying amounts of green or brown, dried trash on the juice viscosity (measured with a special adaptor) are illustrated in Figure 2.

Viscosity at the 25% BT level was significantly higher ($P<0.05$). Significant ($P<0.05$), moderate correlations existed between Brix and viscosity ($R^2=0.68$) and fiber and viscosity ($R^2=0.70$) that indicate % dissolved impurities and fiber from trash contribute to an increase in the viscosity of juices, and most likely the viscosity of boiler house products.

Starch, pH, and Color
Differences in the starch, pH, and color for juices containing varying amounts of either GT or BT are listed in Table 1.

In general, starch levels were relatively low because samples were collected in late season when cane was mature. Starch generally increased with greater amounts of either GT or BT, although there were no significant differences at the 5% probability level. The increase in starch with added BT indicates that brown, dried leaves were still metabolically active, as starch is a temporary storage product of photosynthesis and linked to the metabolism of sucrose. This also showed that not all the starch had been hydrolyzed for plant assimilation. Moreover, this suggests that "dead" leaves is probably a misnomer, and the term "brown, dried" leaves is more factual.
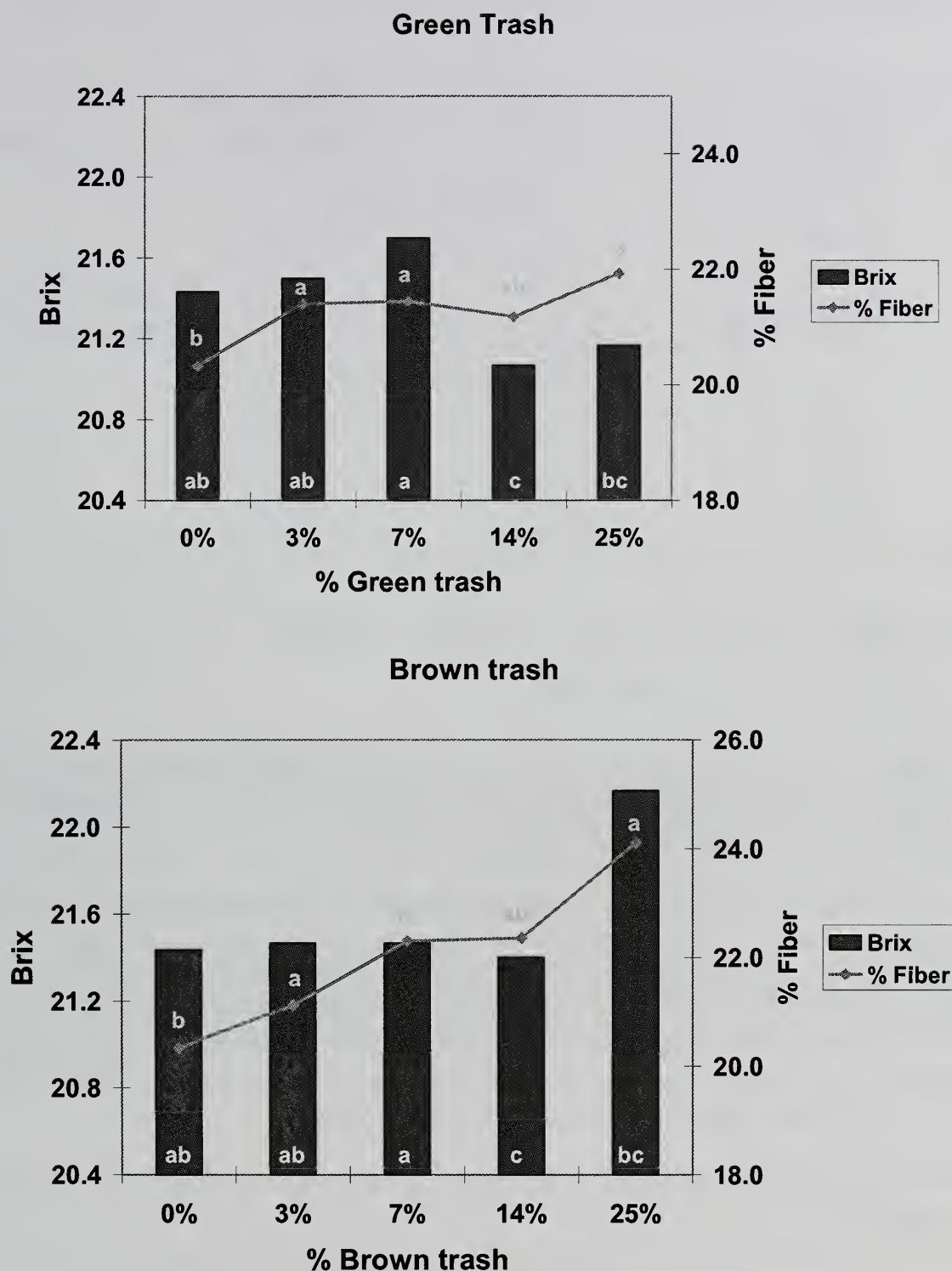
Figure 1. Effect of green and brown, dried trash on the Brix and fiber quality of pressed juice. Letters represent significant differences (P<0.05) among the trash levels separately for Brix and fiber and for each type of trash.
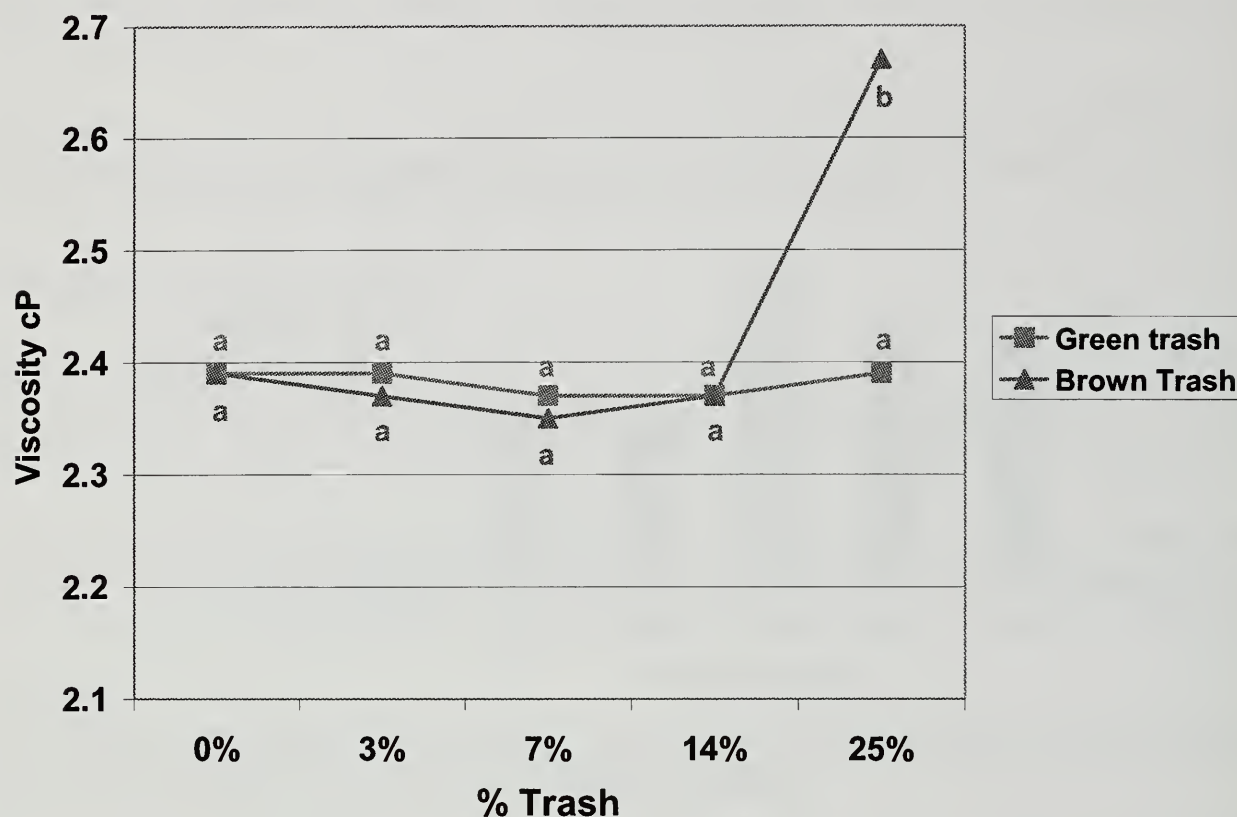
Figure 2. Effect of varying levels of green or brown, dried trash on the viscosity (measured using a special adaptor) of pressed juices. Letters represent significant differences (P<0.05) among trash levels for each separate type of trash.

Juice pHs were generally higher with added BT than GT. This confirms other results by the authors (Eggleston, *et al.*, 2007). Higher pHs may be due to decreased acids, particularly aconitic acid that has been reported to be abundant in leaves and tops (Balch and Broeg, 1948) or the buffering capacity of BT. As there was a strong correlation (R^2=0.85; P<0.05) between pH and Brix (the correlation was slightly weaker at R^2=0.70 for combined BT and GT Brix), it is more likely that the dissolved impurities from BT contained a compound with a relatively higher pH or higher pH buffering capacity, rather than decreased acids.

As expected, color generally increased at the higher levels of GT additions. GT affected juice color more than BT.

Table 1. Differences in the starch, pH and color values for pressed juices with varying amounts of green or brown, dried trash.

Level of Trash in Juice	Average Starch[a] (ppm/Brix)	Average pH[a]	Average Color[b] (ICU)
0% (control)	121.2	5.35	6264ab
Green Trash (GT)			
3% GT	116.6	5.37	6075ab
7% GT	133.8	5.36	5803 b
14% GT	168.8	5.37	6350ab
25% GT	171.7	5.36	6790 a
Brown, Dried Trash (BT)			
3% BT	154.3	5.38	6885 a
7% BT	161.6	5.38	6827 a
14% BT	166.0	5.37	6348 a
25% BT	184.5	5.43	6420 a

[a] There were no statistical differences ($P<0.05$) among starch and pH values for both trash types at various levels of added trash

[b] Lower case letters represent significant differences ($P<0.05$) for color among trash levels for each type of trash only.

Thermal Conductivity and Resistivity

Thermal conductivity is the ratio of heat flux density to a temperature gradient in a material, and indicates the ability of a material to conduct heat. Thermal resistivity is the reciprocal of thermal conductivity and indicates the resistance of a material to conduct heat. Both of these parameters are important to juice heating, clarification, evaporation, and crystallization processes.

Generally, thermal conductivity and resistivity (Figure 3) changed less with the addition of brown, dried trash compared to green trash. This is most likely because BT absorbs more water. However, the small but significant differences on addition of both types of trash may have no effect in the factory.
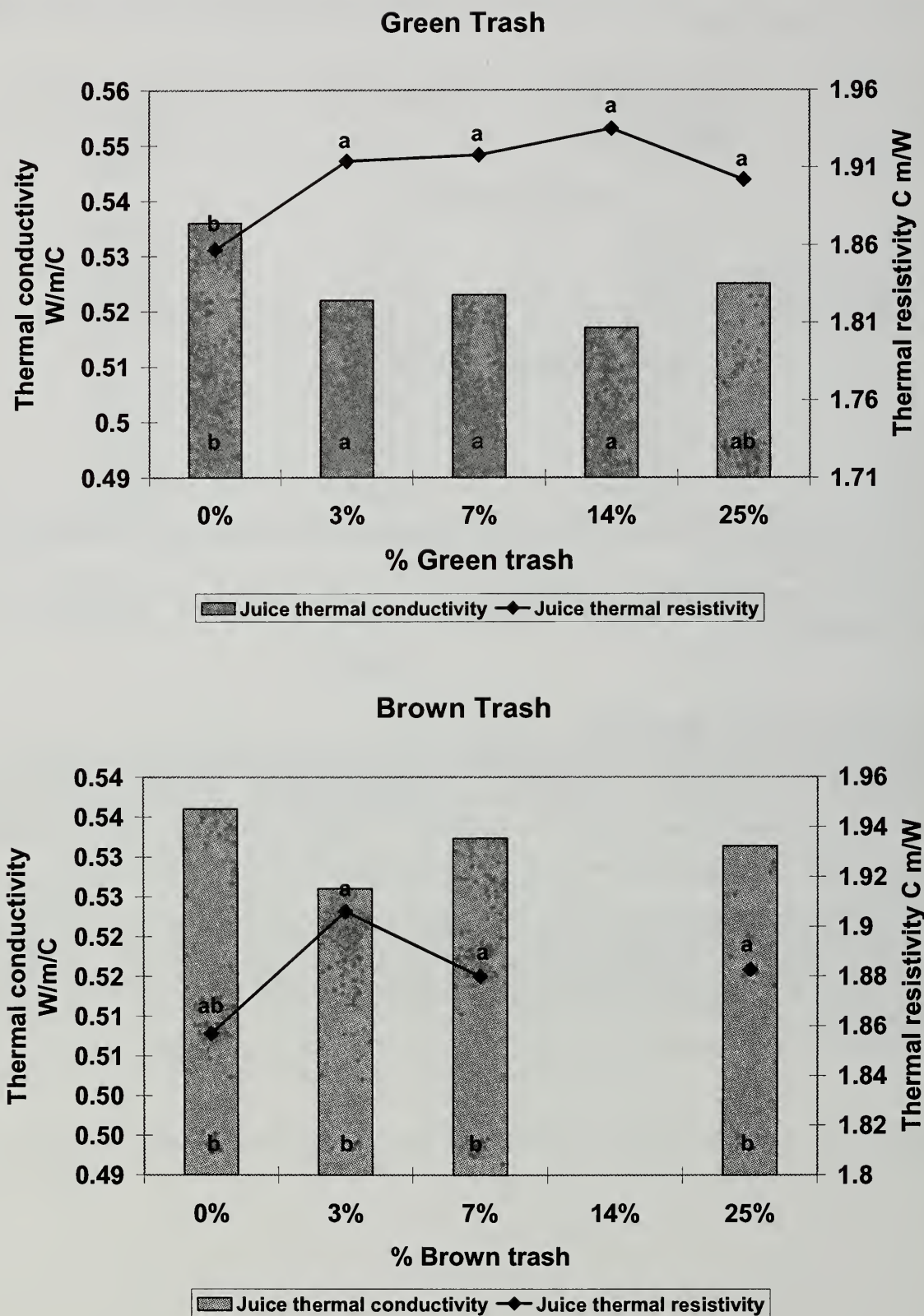
Figure 3. Effect of green and brown, dried trash on the thermal conductance and resistivity characteristics of pressed juice. Letters represent significant differences (P<0.05) among the trash levels separately for thermal conductivity and thermal resistivity and for each type of trash.

Mud Characteristics after Clarification

Juices containing trash were clarified by a hot lime clarification procedure in the laboratory, because this is now the most popular industrial method for clarification of juice in U.S. factories (Eggleston, *et al.*, 2003). In general, larger particles were found in settled mud when 25% trash was added to the juice, although the differences were not great (Figure 4). Cellulosic material was visible in the 25% BT micrograph (Figure 4).
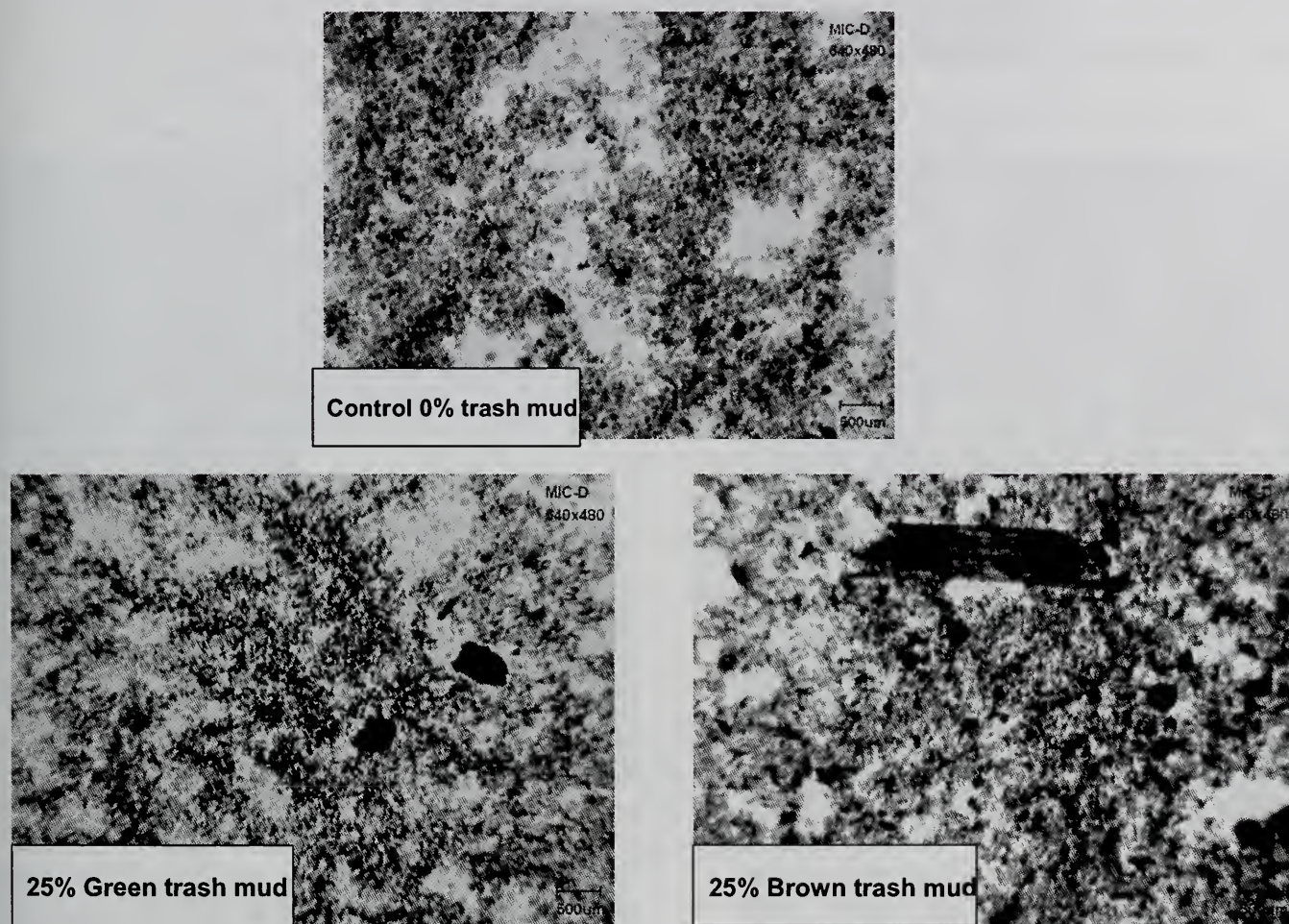
Figure 4. Micrographs of settled mud floc after clarification of juices containing differing amounts of trash.

Clarified Juice Properties

Micrographs of clarified juices from pressed juices containing varying amounts of GT or BT are shown in Figure 5. Marked differences were visible. Generally, clarified juice from juices containing BT had more and larger (up to ~220 µm) microparticles than those containing GT. Screening in the factory will remove some microparticles.
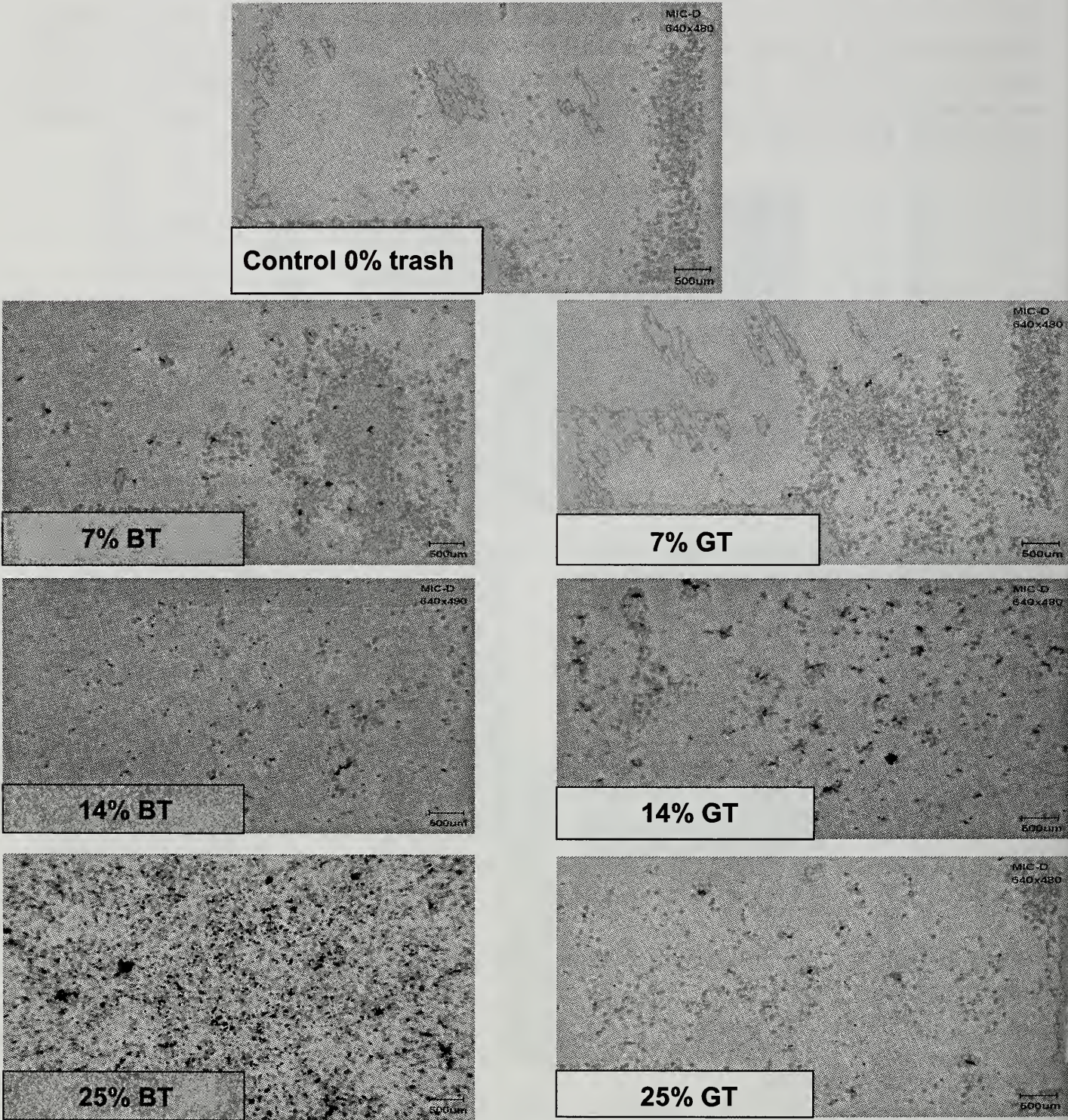
Figure 5. Micrographs of typical unscreened clarified juices from pressed juices containing varying amounts of green or brown, dried trash.

Conclusions

Overall, results from this preliminary study indicate that the detrimental effect of brown, dried trash on factory processing has been previously underestimated. Other conclusions include:

- Fiber amounts increased when both GT and BT were added, but significantly more with BT.
- BT contributes more than GT to the factory delivery of soluble solid impurities, which can increase the viscosity of juice at the 25% BT level.
- Brown, dried trash can still be metabolically active, i.e., not dead, and contain starch.
- Generally, clarified juice from juices containing BT had more and larger (up to ~220 μm) microparticles than those containing GT. Screening in the factory will remove some microparticles.

Future Work

In the factory, the mill operation includes imbibition water and would be expected to pulverize and disintegrate more trash than the extraction method used in this study. Therefore, future work will include further pulverization by using blenders and imbibition water. The effect of weather and deterioration on different types of trash on the processing of juices, syrups, and massecuites will also be evaluated.

Acknowledgements

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Determination of Extraneous Matter and Its Effects on Colombian Sugar Production

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Abstract

Extraneous matter content is one of the factors of great importance associated with the harvesting, loading and transport of sugarcane from the field to the mill. Extensive research conducted in different regions of the world has shown that extraneous matter affects both production and the quality of the end product (sugar). Consequently, one of the objectives proposed by the Colombian agroindustry was the need for evaluation systems that guarantee the rapid, reliable determination of extraneous matter that enters a mill and that identify the best harvesting options, especially for green (i.e., nonburned) cane and for reducing the content of extraneous matter in the cane in the field or delivered to the mill. Sampling systems and control of extraneous matter by means of mechanized core sampling or the manual cleaning of samples obtained at a station for the postharvest evaluation of cane were evaluated for different harvesting methods. Based on the results obtained, it was concluded that harvesting sugarcane with the manually cut, clean green cane procedure (System 1) contributed to reducing the levels of extraneous matter in the cane for milling and to increasing sugar yields. The possibility of using NIR (near infrared spectroscopy) to quantify extraneous matter incorporated in the cane during its cutting, loading, transporting and delivery to the mill is being explored in conjunction with a pilot mill in the Cauca Valley.

Introduction

Extraneous matter is defined as plant matter other than the cane stalks rich in sucrose, which together with mineral matter consisting of dirt and stones, reduces sucrose levels and interferes with the processing of the harvested cane. Plant residue consists of leaves, tops, stools, leaf sheaths, suckers, dry cane and weeds. It is generally accepted that the levels of extraneous matter can be attributed to the harvesting system, the loading of the cut cane and the variety. In the case of the Colombian sugar sector, the levels of green or dry leaves, tops, suckers and dry

cane can be attributed mainly to the harvesting system, the cutter's work and the operation of the harvesting machines.

Chemical analyses of the different plant matter components considered to be trash have shown that in some cases the contribution of sucrose % cane should also be taken into account (e.g., for the tops and suckers, where levels of sucrose % cane range from 2-10%), depending on the season, varieties and harvesting conditions (Table 1). Nevertheless, the incorporation of higher contents of other components such as polysaccharides, potassium salts and color precursors (polyphenols) in the tops and suckers during the harvesting and later processing in the mill versus those of clean stalks could reduce or otherwise affect the recoverable sugar and quality of the end product (sugar). Observations on a laboratory scale have also made it possible to determine that the leaves and dirt have the greatest impact on the sucrose and recoverable sugar content, reducing the recoverable sugar from 0.20-0.30 units (%) for each 1% of these materials included during the harvesting process (Table 2).

Table 1. Characteristics of extraneous matter.

Plant Matter	Sucrose % cane	Fiber % cane
Tops	2 – 6	13 – 20
Suckers	3 – 10	10 – 17
Leaves	---	20 – 50

Table 2. Changes in the content of sucrose and recoverable sugar (% ERS) for different kinds of extraneous matter.

Matter	Sucrose % cane	ERS % cane [1]
Clean cane	15.0	13.0
Tops (1%)	- 0.13	- 0.15
Suckers (1%)	- 0.05	- 0.07
Leaves (1%)	- 0.17	- 0.21
Dirt (1%)	- 0.22	- 0.30

[1] ERS % cane = sucrose % cane – b x nonsucrose % cane – c x fiber % cane, where $b = 0.5$ and $c = 0.057$.

With regard to the impact of extraneous matter on the zero percentage levels of non-sucrose and fiber % cane, it was observed that in trials carried out with variety CC 85-92, both the insoluble mineral matter (dirt) and the extraneous plant matter had similar increases in the non-sucrose: 0.020 units for each 1% of dirt or plant matter. On the other hand, the mineral matter increased the fiber % cane in greater proportion in relation to the components of the extraneous plant matter: an increase of 0.80 in the fiber % cane for each 1% of dirt incorporated in the clean cane (Larrahondo, 1999).

Experimental

Procedures for Determining the Amount of Extraneous Matter
Visits made to the sugar mills in Colombia, where control of extraneous matter is done, indicate that there are two sampling and evaluation systems: mechanized core and claw. During the visits and data gathering, the number of samples per day, the sample size (weight) and average cost were estimated for each method.

To observe the functioning of new methodologies for quantifying the foreign matter that enters the factory, the respective calibrations and validations were initiated, using NIR (near infrared) spectroscopy. For the NIR calibration, 500 g of de-fibered cane mixed with different percentages in weight of plant foreign matter (leaves, tops, suckers, etc.) and mineral matter (1-20%) were used. For each treatment at least two replications were analyzed, and the NIR Foss 6500 equipment was used with reflectance detector readings in the range of 1100-2500 nm. In addition, conventional procedures described for NIR (Burns and Ciurczak, 1992; McDonald-Lewis, 1992; Wetzel, 1983;) were used.

Impact of Extraneous Matter Associated with Different Harvesting Systems
The Colombian sugar industry has increased the green cane harvest area in compliance with its commitment not to burn in restricted areas in line with the Government's clean-production regulations. At present, green cane is being harvested both manually and using mechanization. In the case of the former, there are three options or procedures for the harvest, which are reflected in the levels of extraneous matter that arrive at the mill. In general the systems of manually cut green cane are classified as the conventional green cane system (no cleaning – System 2), and clean green cane (System 3).

From 1998-2002 commercial and noncommercial monitoring was done in the Colombian sugar mills, where the levels of foreign matter and sugar yields were determined for manual systems (2 and 3) as well as for mechanical harvesting (burned and green – Systems 4 and 5, respectively). Similarly, for Systems 2 and 3, comparisons were made with respect to the efficiency of the cane cutter, percentages of foreign matter and yield.

At CENICAÑA, the high leaf-shedding varieties (CC SP 89-1997, CC 91-1999, CC 89-2000 and MZC 74-275, the control) were evaluated at different sugar mills in order to determine the differences in sucrose % cane between the two manual systems (2 and 3).

Measuring the Impact of Extraneous Matter on the Processes of Milling and Preparation. Commercial-Scale Monitoring
With the cooperation of the Manuelita Sugar Mill (considered the pilot), the effects of extraneous matter on the manual and mechanical cutting systems (harvesters CAMECO 9417 and AUSTOF 9415) were evaluated in the factory. The contents of sucrose, brix (total soluble solids), purity, index of preparing the cane, extraction and efficiencies of milling were compared for the two harvesting systems.

On a laboratory scale the clarification of cane juices (obtained using a hydraulic press) that came from samples that had levels of extraneous plant matter (leaves, tops and suckers, and a mixture of the three components) was evaluated in percentages of 0% (clean cane), 5%, 10% and 15%.

Results and Discussion

Procedures for Determining the Amount of Extraneous Matter

A state-of-the-art diagnosis regarding the control of extraneous matter in the Colombian sugar sector identified two sampling and evaluation systems: mechanized core sampling and the use of a manual claw. The latter, which is used in eight mills, can characterize or analyze a maximum of only 55 samples per day. On the other hand, when using the former system, which is used in four Colombian mills, from 115-120 evaluations are done daily with an average sample weight per evaluation of 8.5 kg (Table 3). In a series of simultaneous sampling and analyses (in duplicate) using the two systems for cane harvested manually and mechanized, corresponding to the commercial lots of 10 varieties, there were differences in determining the plant matter content incorporated during both the manual and mechanized cuts (Table 4); however, no significant differences were found for the overall evaluation of extraneous matter for the cane harvested manually. In general it was observed that the manual-claw procedure gave the highest values of total extraneous matter, with a lower value of replicability than for the mechanized core sampling (Table 5).

Table 3. Current systems for sampling extraneous matter in the Cauca Valley (Colombia).

System	No. Mills	No. Samples/Day	Average Sample Wt (kg)
Mechanized core sampling	4	115-120	8.5
Manual claw	8	10 - 55	350

Table 4. Comparison of two procedures for evaluating extraneous matter.

Type of Harvest	Difference Plant Matter[1]	Difference Mineral Matter	Total Difference
Manual	-1.2 (S) [2]	2.2 (NS)	1.0 (NS)
Mechanized	3.9 (S)	1.6 (NS)	5.1 (S)

[1] Difference in the values of extraneous matter determined via the manual claw and mechanized core sampling.
[2] S = Significant difference at a level of 95%.
NS = Nonsignificant difference at a level of 95%.

Table 5. Replicability of two procedures for evaluating extraneous matter.

Method	Plant Matter	Replicability Mineral Matter	Total Extraneous Matter
Manual claw	5.9	17.1	13.1
Mechanized core sampling	5.4	20.9	22.7

Although the claw sampling procedure gives good quantification and characterization of the extraneous matter, it is inconvenient because it is costly, requiring a greater number of workdays (average 5 per shift) in order to evaluate at least 10 commercial lots per day with a good level of replicability. As for the mechanized core sampling system, it requires fewer laborers (2 per shift) and can handle a higher number of samples, but its replicability is not as good as the claw procedure (Table 5). Given the foregoing, the replicability of these two procedures will have to be improved, or studies will have to be conducted to determine how to reduce the costs of these evaluations while maintaining a highly reliable method for a large number of samples.

NIR spectroscopy has arisen recently as a new and better alternative for evaluating and quantifying the extraneous matter content that enters a mill. This methodology is based on a sampling of de-fibered cane that is taken before the first milling. The results reported by Brotherton and Berding (1995) and Staunton et al. (1999) in Australia, as well as the NIR calibrations carried out recently at CENICAÑA (Larrahondo, 2001), using the mechanized core sampling system with manual cleaning as the primary method indicate that this spectroscopic method has good potential (Table 6).

Table 6. Results of the calibration and validation for determining extraneous matter content.

Determination (%)	Range (%)	Method	Calibration R	SEC	Validation R	SEP
Clean cane	0 – 61	PLSR 2nd deriv.	0.93	3.3	0.84	3.9
Extraneous plant matter	0 - 24	PLSR 2nd deriv.	0.93	2.4	0.80	2.4
Extraneous mineral matter	0 – 15	PLSR 2nd deriv.	0.92	1.7	0.86	2.1
Total extraneous matter	0 – 39	PLSR 2nd deriv.	0.93	3.3	0.85	4.1

Impact of Foreign Matter Associated with Different Harvesting Systems
With manual cutting the mills normally register levels of extraneous matter from 1.8 – 3.7% in burnt cane (System 1); from 2.7.7.7% in non-cleaned green cane (system 2); and from 1.5-3.0% in the clean green cane (System 3). When the harvest is mechanized, extraneous matter is around 9.5% in burnt cane (System 4) and over 10% in green cane (System 5). The data also indicate

higher yields in sugar with the manual, clean green harvest as compared to the other options (Table 7) (Villegas, 2002).

Table 7. Extraneous matter and yield in sugar (% cane) by harvest system and procedure (nine mills).

Variable	System 1	System 2	System 3	System 4	System 5
Extraneous matter	3.3	5.9	2.6	9.5	12.4
Sugar yield (%)	11.8	11.1	12.1	11.6	10.9

System 1: Manual harvest – Burnt cane
System 2: Manual harvest – Green cane
System 3: Manual harvest – Clean green cane
System 4: Mechanized harvest – Burnt cane
System 5: Mechanized harvest – Green cane

In the manual harvests the difference in yield in favor of the clean green cane is approximately 0.5 and 0.8 percentage points as compared to the burnt cane. Similarly, the yields with clean green cane can reach 1.5 percentage points higher than with non-cleaned green cane of the mechanized harvest.

The commercial data on the cane harvested at a Colombian sugar mill during 1998 (Table 8) confirmed these results and showed that in the clean green cane, extraneous matter was reduced to 1% versus 6% for the conventional green cane harvest and 10% for the mechanized green. The commercial yield of sugar with the cut clean green cane was 2 points higher than the mechanized green and the conventional manual green harvest (Viveros et al., 1999).

Table 8. Comparison of the different harvesting systems in green cane (1998).

Variable	System 5	System 2	System 3
Average cutter efficiency (t/man/day)	---	3.0	1.5
Extraneous matter (%)	10.3	6.0	1.0
Sugar yield (%)	10.3	10.0	12.0

In System 3 the cutter removes the leaves and cuts the stalk at the base, removes the top and stacks the cane in the field. He also carries out a complete cleaning of the piling/loading zone. Thus this harvesting system has a higher cost than System 2, which could be a limitation for its adoption. To reduce this extra cost, it is necessary to increase the cutter's efficiency through actions that facilitate his work such as planting varieties that shed leaves, where it is easy to remove the leaves attached to the stalks and that are resistant to lodging. CENICAÑA varieties

selected for self-trashing was found, for example, that variety CCSP 89-1997 in the procedure for System 3 had an efficiency of 3.2 t/man per day, while variety CC 89-2000 had a yield of 2.5 t/day, for an efficiency 3.9% higher than the average obtained with the commercial varieties for System 3. Similarly, among the varieties evaluated, the difference in sucrose (% cane) ranged from 0.4-2.1 percentage points in favor of System 3 (Table 9).

Table 9. Differences in sucrose (% cane) from clean green cane (system 3) and conventional green (system 2) harvesting systems for different varieties in Colombia.

| Variety | Sucrose (%)[1] | | | |
	Mill A	Mill B	Mill C	Mill D
CCSP 89 - 1997	1.7	0.4	1.7	0.8
CC 91 - 1999	2.1	1.4	0.5	1.7
CC 89 - 2000	0.7	0.8	1.3	0.9
MZC 74-275	1.7	1.5	0.6	1.0
Average (X)	1.5	1.1	1.0	1.2

[1] Differences in sucrose from Systems 2 & 3 in favor of the latter.

Measuring the Impact of Extraneous Matter in the Processes of Milling and Preparation. Commercial-Scale Follow-Up.
In a commercial-scale follow-up done with cane variety CC 85-92, using two harvesting systems (manual and mechanical cutting), significant differences were found between the two harvesting systems with respect to the starch and dextran in the juices (first extraction and diluted) and molasses. The highest values of these parameters corresponded to materials from the sugar process from the mechanical harvest, which had an average increase of 11 percent units of extraneous matter compared with the manual cut (Table 10).

In clarification trials on a laboratory scale, a notable increase was found in turbidity and color of the clarified juice under the conditions of pH 7.0, temperature of 97° C and 6 and 10 mg/l of flocculant when the levels of extraneous plant matter in the cane (primarily leaves and shoots) were increased.

Table 10. Color, starch and dextran in diluted juices and molasses observed in two harvesting systems (26-30 April 2003), Manuelita Mill. Variety CC 85-92.

Harvesting Systems	N	Extraneous matter	Diluted Juice			Syrup		
			Color $(UI \times 10^3)$	Starch (%, brix)	Dextrans (%, brix)	Color $(UI \times 10^3)$	Starch (%, brix)	Dextrans (%, brix)
Manual cutting (green cane)	10	5.1	8.9 b	0.07 b	0.03 b	9.7 b	0.22 b	0.08 b
Mechanical cutting	13	16.4	12.5 a	0.12 a	0.51 a	11.5 a	0.35 a	0.52 a

a, b: Equal letters means no significant difference between average values for each column:

N: Samples number (1 composite sample / h)

Conclusions

- Extraneous matter per day of milling is higher for Systems 5 and 2, which suggests that for the sugar industry, System 3 is an alternative that can reduce the difficulties of milling and the sucrose losses in the mill.

- Despite the fact that System 3 requires more cutters, this can represent a socio-economic benefit by providing work for unskilled labor.

- With respect to the R&D needs, the demand for varieties that facilitate the harvesting of green cane, both manually and mechanized, should be one of the objectives of a breeding program for supplying cane of optimum quality to the factories. The selection of self-trashing varieties not only greatly favors harvesting System 3 but also increases the efficiency of the cutter per day.

- The establishment of a standardized methodology for determining extraneous matter in the cane to be milled, with good precision and a larger number of analyses per day is another of the demands for R&D. At present NIR spectroscopy is a good technological option, which is in the process of development, commercial validation and adoption.

- The extraneous matter incorporated with the cane not only affects the final recovery of the sucrose, but it also increases the levels of color, reducing sugars, polysaccharides (dextran and starch) and other non-sucrose in the juices and materials, which affects the factory process, the costs and the quality of the final sugar.

Acknowledgements

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Use of Magnesium Compounds and Soda Ash in Cane Juice Clarification – Laboratory Studies

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Abstract

The use of magnesium compounds in clarification, either as a replacement for lime or as an adjunct to lime, has been considered in the sugar industry. In a series of laboratory experiments, lime, magnesium oxide, dolomitic hydrate, dolomitic quicklime, soda ash, and combinations with soda ash were examined for their effect on turbidity, color, polysaccharides, organic acids, purity, invert, settling rate, calcium and magnesium in cane juice. All combinations with calcium (lime, dolohydrate and dolomitic quicklime) decreased juice color, while clarification with magnesium alone or soda ash alone and in combination, increased color. Magnesium compounds and soda ash have potential in juice clarification, especially when there is a need to control calcium, but lime is generally superior in removing color, turbidity and polysaccharides.

Introduction

In processing cane juice, the most important step is that of lime clarification. Lime addition has two main beneficial effects: It neutralizes and stabilizes the pH of the juice, helping to prevent sucrose loss due to acid inversion, and it precipitates soluble and insoluble impurities.

The beet industry uses much higher levels of lime in processing than does the cane industry (1.2-2% lime per ton of beet vs. 0.05% lime per ton of cane), so there is not such a disposal problem for cane as there is in the beet industry. Nevertheless, some difficulties associated with calcium in the cane industry are increased evaporator scale, slowing of crystallization, and a moderate melassigenic effect, resulting in loss of sucrose to molasses.

High calcium levels were observed in studies of the "hard-to-boil" phenomenon that occurs periodically in Louisiana, with indications that excess calcium may play a role in this problem. [1] This is especially so when extra lime is added for juice clarification due to high mud levels. All of these factors show that control of calcium levels is important for the cane sugar industry.

Two processing adjuncts have been considered as aids in controlling the amount of lime added during juice clarification, with some consideration given to the idea of completely replacing lime with them, namely, magnesium salts or soda ash.

Magnesium Salts

Magnesium compounds were examined in Hawaii, Mauritius, Jamaica and Puerto Rico in the 1960s and in the beet industry in the 1980s, with generally good results. Reported advantages of using magnesium included a more rapid settling rate, smaller volume of settling, less evaporator scale formation, improved evaporator performance, and less melassigenic action than calcium, resulting in less molasses production. The fact that magnesium compounds are more expensive than lime and usually need to be transported farther has tended to discourage their widespread adoption.

There have been recent reports that some mills in Brazil are using dolomitic quicklime instead of lime in clarification. [2,3] Observations were that magnesium helped to reduce the formation of scale and minimized sucrose loss.

Three magnesium salts have potential in juice clarification: Dolomitic quicklime, dolomitic hydrate and magnesium oxide.

Dolomite is a calcium-magnesium carbonate ore, which, when heated, forms oxides. The resulting product, called burnt dolomite, is a mixture of calcium oxide and magnesium oxide (54-65% CaO and 35-46% MgO), which is also known as dolomitic quicklime. When hydrated, dolomitic hydrate is formed, a mixture of calcium and magnesium hydroxides, sometimes called dolohydrate.

According to literature from Premier Chemical Company, Magox ® is a high performance alkaline magnesium oxide exhibiting approximately 1.85 times the neutralizing power of high calcium hydrated lime. Therefore, only about 54 percent as much Magox® needs to be used as lime for total replacement. The company (then known as Basic Chemicals) conducted a series of tests in the 1960s on replacement of lime in Hawaii, Mauritius, Reunion, Peru, Jamaica, Puerto Rico, Antigua, Mexico, Florida and Louisiana, with very satisfactory results (company literature). In order to fully hydrate, magnesium oxide requires some pressure or a longer time, so its preparation is more complicated.

Magnesium has a slightly lower melassigenic effect than calcium and sodium has about double the melassigenic effect. [4,5]

Soda Ash

Soda ash (sodium carbonate, carbonate of soda, Na_2CO_2) is routinely used in the beet industry when beets are deteriorated because of its effect on calcium solubility. According to Carlson [6], it both reduces juice calcium and increases alkalinity. Calcium salts cause difficulties in beet juice boiling and addition of soda ash is routinely recommended in the beet sugar industry. [7] In older literature, soda ash was described as useful for neutralizing excess acidity in deteriorated cane juice, for more complete precipitation of lime, and to decrease viscosity of massecuites. [8]

Materials and Methods

Mixed juice was heated to 85°C, the clarification agent was added at a level consistent with lime use in the cane industry, then a commercial flocculent was added, and the juice stirred slowly for about 3 min. The juice was then decanted into a graduated cylinder and the settling rate (ml) was observed over 30 minutes. Treated and untreated juices were tested for color, polysaccharides, turbidity, organic acids, magnesium, calcium, purity, invert and settling rate.

Midland PCS-3000 flocculent was used, prepared according to the company's technical bulletin and applied at the rate of about 5 ppm on juice.

Compounds tested in this study included Magox® (magnesium oxide) provided by Premier Chemical, LLC, dolohydrate/dolomitic hydrate (a mixture of calcium and magnesium hydroxides) provided by Chemical Lime Company, dolomitic quicklime (mixture of calcium and magnesium oxides), provided by Chemical Lime Company, and 50:50 combinations with soda ash. Details are shown in Table 1.

Limed juice and heated juice were used as controls. The effect on settling rate, pH, color, turbidity, polysaccharides, sucrose, invert, organic acids and cations (Ca and Mg) was determined. All data shown represent the average of two experiments.

Table 1. Compounds tested.

Compound name(s)	Symbol	Composition
Lime	L	$Ca(OH)_2$
Dolohydrate; dolomitic hydrate	DH	50% $Ca(OH)_2$ + 50% MgO
Dolomitic quicklime	DQ	55-80% CaO + 20-42% MgO
Magnesium oxide; Magox®	MgO	93.7% MgO
Soda Ash	SA	Na_2CO_3
Lime + Soda Ash	L/SA	50% of each
Dolohydrate + Soda Ash	DH/SA	50% of each
Dolomitic quicklime + Soda Ash	DQ/SA	50% of each

Results and Discussion

Table 2 shows the settling rate of the various clarifications agents. The limed juice settled most slowly and had smaller precipitant particles than the juice treated with magnesium or soda ash. Magnesium oxide (Magox®) settled most rapidly, but it did not clarify as well and had increased color. The settling of magnesium oxide was dramatic, with the majority of settling activity occurring

in less than one minute. Soda ash, DH/SA mixture, and DH had similar settling rates and were the next fastest to settle. The settling rates are shown graphically in Figure 1.

Table 2. Settling rate of various clarification agents. Vol (ml) of precipitate over time (min).

Time	Lime	DoHy	DoQL	MgO	S.A.	Lime/SA	DoHy/SA	DoQL/SA
1	53	31	45	16	30	37	24	39
5	33	23	27	12	21	19	17	22
10	21	18	22	12	17	17	14	17
30	16	15	16	12	13	16	13	16
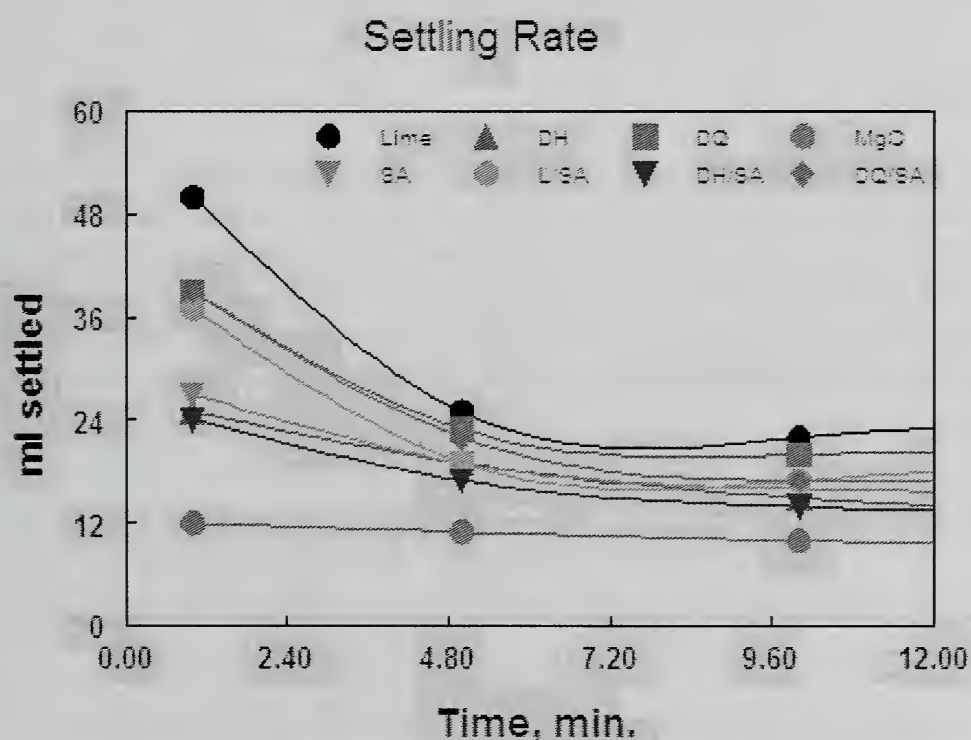
Figure 1. Settling rate of clarification aids in treated cane juice.

Table 3 shows the effect of the clarification aids on pH, purity, and invert. Purity increased slightly (about 1 point) with all of the agents, while glucose and fructose remained stable, indicating that there was little or no loss of sucrose nor destruction of invert. The pH range was somewhat wide (6.23 for Magox® to 9.34 for dolomitic quicklime). Further work should be done to determine the optimal pH range for magnesium salts. It should be noted that in the factory, pH seldom exceed 8.2 and is usually lower.

Table 3. Effect of clarification aids on cane juice pH and purity

Sample	pH	Purity	Glucose	Fructose	Invert
Mixed juice	5.57	88.14	2.07	1.99	4.06
Mixed juice/heat	5.52	88.82	1.98	1.97	3.95
Lime	9.03	89.47	1.88	1.80	3.68
Dolohydrate	8.53	89.14	1.88	1.84	3.72
Doloquicklime	9.34	89.10	1.91	1.78	3.69
Magox	6.23	89.15	1.98	1.92	3.90
Soda Ash	7.09	89.62	1.93	1.84	3.77
Lime/Soda Ash	7.56	89.35	1.88	1.93	3.81
Dolohydrate/Soda Ash	7.41	89.45	1.97	1.96	3.93
Doloquicklime/Soda Ash	7.95	89.04	1.88	1.88	3.76

Table 4 shows the effect on color, turbidity, polysaccharides and oxalic acid. Oxalic acid is an important component of scale, and its removal during clarification is a benefit. The results showed the strong effect of heat on color, turbidity and polysaccharide. During clarification, heat alone conferred most of the advantage in removal of turbidity (85.3%) and polysaccharides (29.6%), but had no effect on oxalic acid. Color was increased by soda ash and all mixtures with soda ash. Magnesium oxide also showed some color increase. None of the aids were as good as lime for removing color from the juice.

Table 4. Effect of clarification aids on cane juice constituents.

Sample	Color (% change)	Turbidity (% removed)	Polysaccharides (% removed)	Oxalic acid (% removed)
MJ/Heat	+13.2	85.3	29.6	0
Lime	- 12.5	96.8	38.4	84
Dolohydrate	- 4.7	95.0	37.3	81
Doloquicklime	- 2.1	96.9	34.0	88
Magox	+12.4	85.4	29.9	58
Soda Ash	+18.2	94.8	33.4	66
Lime/Soda Ash	+6.5	92.9	31.6	84
Dolohydrate/SA	+14.9	92.8	36.5	76
Doloquicklime/SA	+14.1	95.9	38.8	78

Figure 2 shows the pattern of color removal by the clarification agents. Figure 3 shows the removal of oxalic acid by the clarification agents. Figure 4 shows the chromatogram of organic acids in the control juices (raw, heated, and heat + flocculant), showing that none of these treatments removed oxalic acid. Figure 5 shows the effect of dolomitic hydrate on organic acids. Of the organic acids in cane juice, only oxalic acid was significantly affected by the clarification agents.
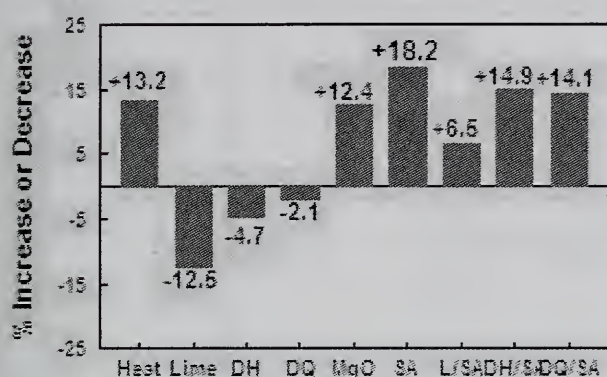
Figure 2. Color removal by clarification agents.
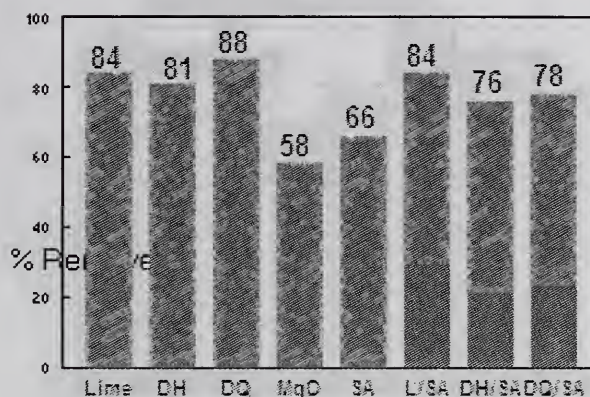
Figure 3. Effect of clarification agents on oxalic acid in cane juice.
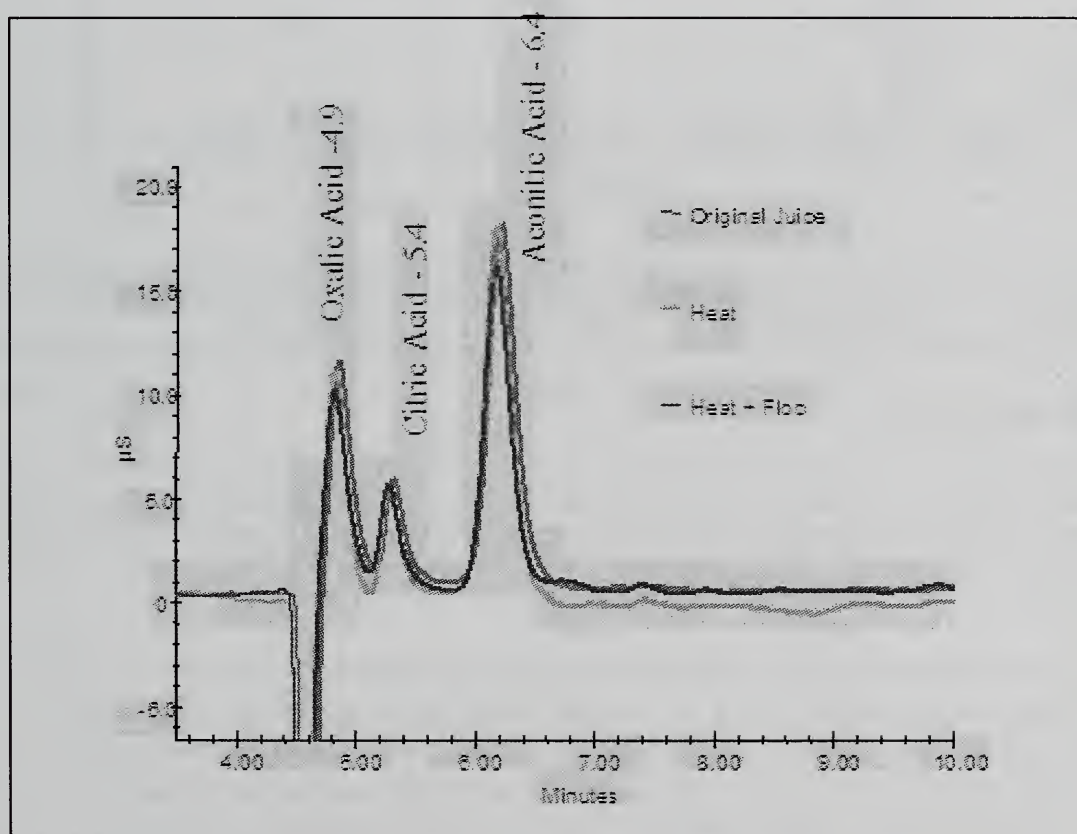
Figure 4. Organic acids in cane juice controls. Neither heat nor the flocculating agent affected the organic acids in the cane juice.
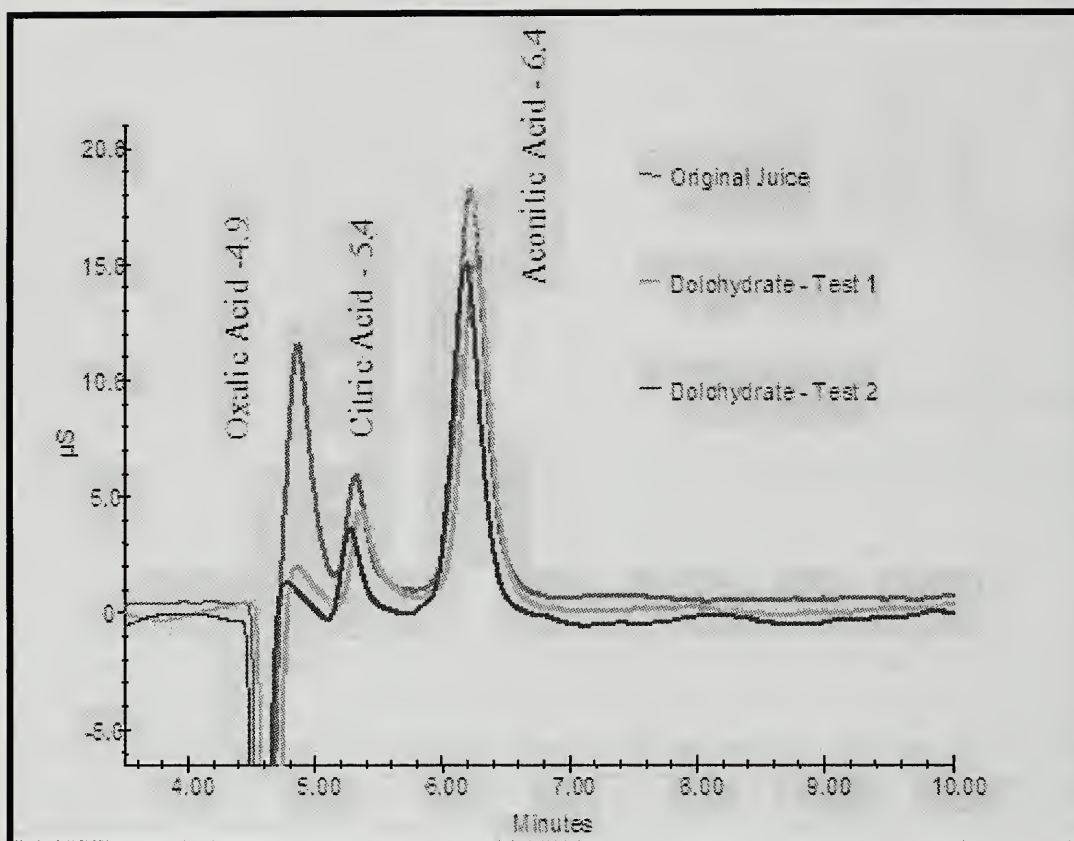
Figure 5. Effect of dolohydrate (dolomitic hydrate) on juice, showing significant elimination of oxalic acid.

Calcium and Magnesium

Table 5 shows the effect on calcium concentration in juice by the various clarification agents and Table 6 shows the effect on magnesium content. Figure 6 shows the effect on calcium in graphic form, and Figure 7 shows the effect on magnesium.

It was noted that both calcium and magnesium are high in Louisiana cane juice. The juice used in these experiments contained 1587 ppm calcium and 1531 ppm magnesium. The ability of soda ash to remove calcium and magnesium was confirmed. Soda ash removed 51.5% of the native lime in the juice and almost 20% of the juice magnesium. In combinations with dolohydrate and dolomitic quicklime, soda ash was able to assist in removing calcium and magnesium, even though dolohydrate and dolomitic quicklime alone increased both calcium and magnesium, although the latter only by an insignificant amount. Liming removed 33% of the juice magnesium but significantly increased juice calcium. Magox® removed 30% of juice calcium.

Table 5. Effect on calcium levels in juice (ppm on solids).

Sample	Ca added	Total Ca	Ca remaining	% change*
Mixed Juice, heated	0	1587	1587	0
Lime	3000	4587	3133	+97.4
Dolohydrate	1800	3387	1977	+24.6
Doloquicklime	3267	4854	2552	+73.4
MgO	0	1587	1110	-30.1
Soda Ash	0	1587	769	-51.5
Lime + S.A.	1500	3087	1564	-1.4
Dolohy + S.A.	900	2487	1254	-21.0
Doloquick + S.A.	1634	3221	1396	-12.0

* Change (%) in calcium after clarification, compared to amount initially in juice (1587 ppm).

Table 6. Effect on magnesium levels in juice (ppm on solids)

Sample	Mg added	Total Mg	Mg remaining	% change*
Mixed Juice, heated	0	1531	1531	0
Lime	0	1531	1025	-33.1
Dolohydrate	1000	2531	1644	+7.4
Doloquicklime	1267	2798	1564	+2.2
MgO	4000	5531	1932	+26.2
Soda Ash	0	1531	1229	-19.7
Lime + S.A.	0	1531	1212	-20.8
Dolohy + S.A.	500	2031	1468	-4.1
Doloquick + S.A.	634	2165	1447	-5.5

* Change (%) in magnesium after clarification, compared to amount initially in juice (1531 ppm).

Effect of Clarification Agent
On Calcium Level in Juice

Figure ~~Effect of clarification agents on calcium concentration of cane juice~~
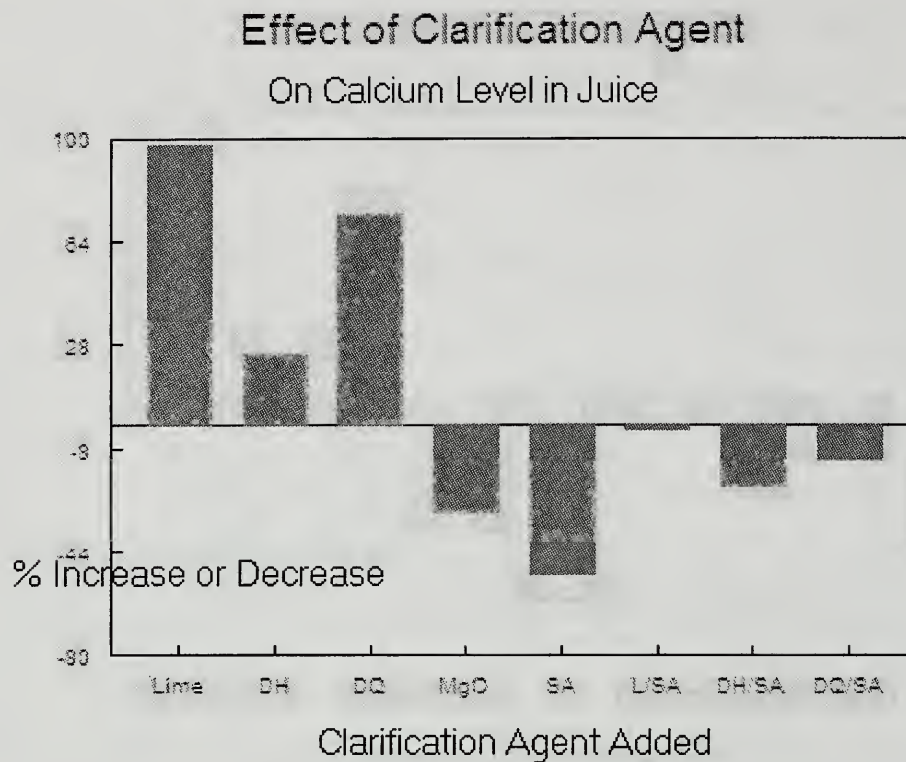
Effect of Clarification Agent
On Magnesium Level in Juice
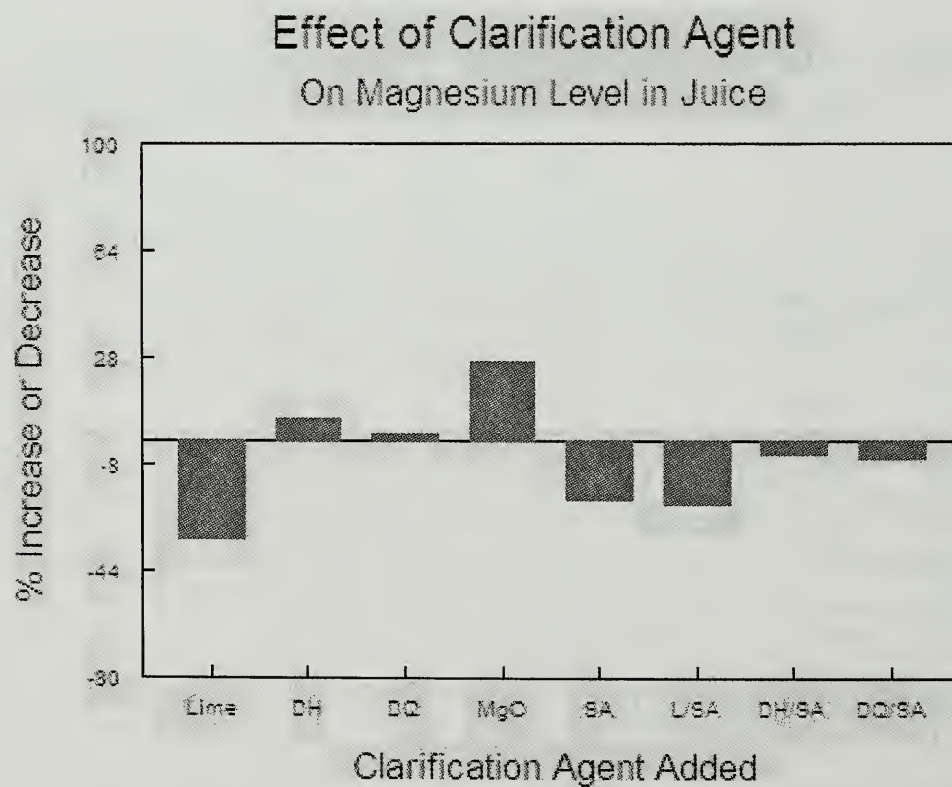
Figure 7. Effect of clarification agents on magnesium concentration of cane juice.

Summary

The following observations were made:

1. All clarifying agents showed approximately 1% purity increase and no sucrose loss, plus a slight decrease in invert.

2. Magnesium oxide settled most quickly but removed less turbidity and increased color. Lime settled most slowly. The other agents and combinations settled at intermediate rates between lime and magnesium oxide.

3. Soda ash alone and in all combinations increased color.

4. Soda ash alone removed 50% of calcium and 20% of magnesium naturally present in juice. In combination with calcium or magnesium salts, soda ash also improved removal of these cations.

5. Lime removed more color and turbidity than the other agents.

6. The best removal of oxalic acid was with dolomitic quicklime.

7. The best removal of polysaccharides was by the dolomitic quicklime/soda ash combination, but it was not significantly different from the effect of lime or dolomitic hydrate.

Conclusions

In conclusion, this study has quantified the effect of various magnesium salts, lime and soda ash in clarification of cane juice. Dolomitic hydrate and dolomitic quicklime show promise as clarification aids - possibly in combination with lime. Magnesium clarifiers tended to settle more rapidly than lime. The sucrose in the cane juice was stable in all of the experiments. The role of heat in purification was shown, and the potential of soda ash as a possible adjunct to clarification was shown because of its ability to facilitate the removal of calcium and magnesium from juice. However, soda ash invariably increased cane juice color and it is highly melassigenic, so use of soda ash should be judicious and applied only when needed. Although magnesium oxide did not perform as well as was expected based on the literature, it is possible that the Magox® had not been sufficiently hydrated for best performance, and further studies should be done to optimize its use. Utilization of magnesium-rich lime salts should be of benefit to the cane sugar industry.

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Laboratory and Mill Studies on the Action of Polyaluminum Coagulants in Cane and Beet Juice

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Abstract

Aluminum-based compounds have a long history of use in purification in water and other industries. A report in 1999 indicated their potential for additional decolorization in raw sugar refining. SPRI conducted a series of investigations on cane and beet juice with commercial polyaluminum coagulants used in water treatment. There was a dramatic increase in removal of color, turbidity and polysaccharides from cane juice compared to traditional lime clarification. Beet juice showed less effect. A mill experiment carried out in late 2005, using two of the commercial polyaluminum coagulants, showed improvements in removal of turbidity, color and ash, and an increase in pH, when compared to traditional clarification. Polysaccharide concentration was not improved. Analysis of treated samples showed no carryover of aluminum in the clarified juice. These preliminary results suggest the potential for polyaluminum coagulants in juice clarification, with the need to optimize the point of addition.

Introduction

Aside from being the earth's most abundant element, aluminum is of interest for a number of reasons. Aluminum-based compounds have long been used to purify water and to remove color and sediment in waste water from other industries. Aluminum compounds are used to remove organics, phosphate, color, iron and suspended particles from water. Aluminum sulfate and polyaluminum chloride (PAC) are the primary chemicals used to treat drinking water. Alum (aluminum potassium sulfate dodecahydrate) and polyaluminum sulfate are likewise primary chemicals used to prepare potable water. Aluminum sulfate is used in the yeast industry to remove phosphate and color from molasses waste water after fermentation, to make it acceptable for discharge into waterways. Aluminum oxides are scavengers of phosphate and silicate. There are reports of the ability of aluminum to remove arsenic and fluoride from groundwater. Aluminum chlorohydrate is used around the world as an alternative to lead clarification in polarization of sugar solutions.

Use of aluminum in sugar processing is not widespread. A report on the potential of aluminum compounds for raw sugar decolorizing was published in 1999.[1] A 1999 patent described the use of PAC to decolorize sugar solutions, sugar alcohols and betaine.[2] It was recently reported that PAC has been used in Brazil at the level of 400-800 ppm to produce direct consumption white sugar to avoid sulfitation [3] and also in India for the same purpose at the level of 1000 ppm.[4]

There exits a need to improve raw sugar quality because refiners continue to require higher quality raw sugar. A major area of potential improvement in raw sugar manufacture is clarification, where the factory should be able to achieve additional removal of color, turbidity and polysaccharides.

SPRI conducted a series of investigations with commercial polyaluminum coagulants used in water treatment. Two were composed of only aluminum compounds and the others were a blend of cationic aluminum polymers with polyquaternaryamine (polyamine). They showed a dramatic increase in the ability to remove color, turbidity and polysaccharides from cane juice relative to traditional lime clarification. Similar studies on beet juice were conducted. The good laboratory results on cane juice led to a mill study in late 2005 in Louisiana.

Regulatory status of aluminum. Up to 1000 ppm Al is permitted for waste water treatment. Aluminum in drinking water is not to exceed 0.2 ppm. PAC are often mixed with polyquaternary amines (polyamine), and mixtures with polyamine are regulated by the polyamine content (21CFR173-60), based on sugar solids. PAC is not specifically approved for use in sugar processing.

Composition of aluminum compounds studied. Table 1 shows the composition of the compounds that were studied. The aluminum compounds were provided by Southern Water Consultants, Inc., Decatur, Alabama. Their formulations are proprietary so only basic formula information was available. The general formula of PAC is: $[Al_2(OH)_xCl_{6-x}]_y$. All are NSF certified.

Table 1. Description of aluminum coagulants used in clarification studies.

Compound	General composition	Max use in potable water
EC 309 HB	Blend of cationic aluminum salts; lightly polymerized.	200 ppm
EC 409	Aluminum chlorohydrate.	250 ppm
EC 461	Blend of cationic aluminum polymers with ~65% polyquaternaryamine.	31.7 ppm
EC 462	Blend of cationic aluminum polymers with ~50% polyquaternaryamine.	41.7 ppm
EC 463	Blend of cationic aluminum polymers with ~25% polyquaternaryamine.	87 ppm
EC 464	Blend of cationic aluminum polymers with ~10% polyquaternaryamine.	100 ppm

Materials and Methods

Modified jar tests were conducted on raw mixed cane juice obtained from a Louisiana mill and from beet juice shipped from a beet processor. Juices were maintained frozen until used. Reduced volumes were used in the jar tests because of the limited amount of juice available at any one time for the experiments.

Jar test: 100 ml raw mixed cane or beet juice was heated to about 80° C and limed to approximately pH 7.3 with constant stirring. A commercial polyacrylamide flocculent used in Louisiana (Midland PSC-3000, 5 ppm) was added, followed by approximately 150 ppm (on juice) of polyaluminum compound. Stirring was continued long enough for complete mixing and the solution then poured into a 100-ml graduated cylinder. The rate of settling at 1, 5, 10 and 30 min was observed. Clarified juice was tested for removal of turbidity, color and polysaccharide, aluminum carry-over and inversion of sucrose.

Several combinations of treatment that included heating the juice and treating with PAC only; or heating the juice and treating with PAC and flocculent, but no lime, were also conducted on cane juice. A set of experiments using soda ash for pH adjustment without lime was also done.

The control juice consisted of juice that was heated, limed and treated with flocculent.

Aluminum analysis: Residual aluminum was determined in juice samples by digesting 2 ml of juice using the nitric acid/hydrogen peroxide procedure of EPA and measuring aluminum by inductively coupled plasma (ICP). In 2005, after destruction of the ICP equipment by the flooding caused by Hurricane Katrina, a colorimetric method based on xylenol orange was used to determine residual aluminum content.[5, 6] Samples were digested as above.

Results and Discussion

Cane Juice.
Table 2 shows the results for cane juice. The results showed that all of the aluminum compounds significantly improved removal of color, turbidity and polysaccharide compared to the control, with the PAC/polyamine combinations having the best performance.

It is noted that EC462 and EC 463 in all cases had significantly better performance than any other treatments. This was true even when neither lime nor flocculent were added and even when soda ash was substituted for lime. While the aluminum-only compounds (EC309 HB and EC409) gave significantly better results than the control, it was evident that better color reduction was obtained with the PAC containing polyamine, and that the added color removal was proportional to the amount of polyamine in the formulation.

The results without lime and with soda ash instead of lime indicate that use of PAC/polyamine combinations have the potential to reduce or eliminate lime usage.

Table 2. Results of treating cane juice with cationic polyaluminum preparations. The highlighted rows show the best action within treatment groups.

Treatment	% Removed from Cane Juice by Treatment		
	Color	Turbidity	Polysaccharides
Heat only	10.8	85.6	27.2
Heat, Lime, Flocculent* (control)	34.2	96.9	35.3
Heat, lime, flocculent + EC 309 HB	51.2	99.1	47.6
Heat, lime, flocculent + EC 409	60.5	99.5	47.8
Heat, lime, flocculent + EC 462	73.0	99.6	54.4
Heat, lime, flocculent + EC 463	75.5	99.5	56.1
Heat, lime, flocculent + EC 464	68.6	99.5	52.1
Heat + EC 309 HB + Floc (no lime)	26.5	98.1	43.5
Heat + EC 409 + Floc (no lime)	35.9	98.3	48.2
Heat + EC 462 + Floc (no lime)	60.9	97.5	56.4
Heat + EC 463 + Floc (no lime)	50.6	98.6	50.1
Heat + EC 464 HB + Floc (no lime)	44.2	98.8	52.1
Heat + EC 309 HB only (no lime, no floc)	28.1	98.2	43.3
Heat + EC 409 only (no lime, no floc)	32.1	97.2	48.5
Heat + EC 462 only (no lime, no floc)	52.3	97.5	52.7
Heat + EC 463 only (no lime, no floc)	48.9	98.0	51.2
Heat + EC 464 only (no lime, no floc)	35.2	95.4	51.1
Heat, soda ash, flocculent + EC 309 HB *	23.7	97.8	44.4
Heat, soda ash, flocculent + EC 409	26.0	98.0	45.3
Heat, soda ash, flocculent + EC 462	62.4	99.2	56.9
Heat, soda ash, flocculent + EC 463	53.1	99.6	55.9
Heat, soda ash, flocculent + EC 464	46.8	99.4	50.7

* Soda ash alone increases juice color by 15-18%.

Settling was not as good with the aluminum compounds compared to lime clarification, shown in Figure 1. Since the original experiments were performed on juice that had been frozen in the laboratory for preservation, settling was tested on fresh, never frozen juice, and the poorer settling was confirmed.
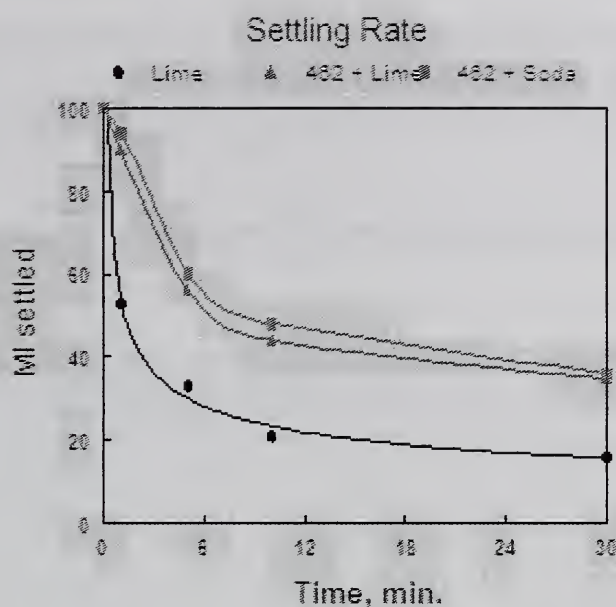
Figure 1. Settling rate of cane juice clarified with aluminum coagulant EC 462, using either lime or soda ash for pH adjustment compared to lime alone.

Residual aluminum. Juice treated with the aluminum compounds was tested for residual aluminum. It would appear that there is less aluminum in the clarified juice after treatment with the aluminum coagulants than is present in the raw juice:

EC 309 HB	1.69 ppm
EC 409	2.07
EC 462	1.89
EC 463	0.41
EC 464	0.71
Raw cane juice	31.9

Beet Diffusion Juice.
The results of treating beet diffusion juice in the same manner as cane juice are shown in Table 3.

In the beet juice, polysaccharides appeared to be re-solubilized by the lime. This effect is clear when comparing heated juice with heat and lime or heat, lime and flocculent (control). Heat alone removed 23% of polysaccharides, but addition of lime resulted in only 3-5% polysaccharide removal. This trend is repeated in the results comparing the series with aluminum coagulants with and without lime. Without the lime, the coagulants efficiency (24.5-32.9% polysaccharide removal) was generally superior to polysaccharide removal compared to when lime was added (3.9-21.6% removal). Lime, however, worked synergistically with the coagulants for improved removal of color and turbidity, the exception being EC 463, which alone exceeded all of the heat-lime-floc test parameters.

It was notable that aluminum treated beet juice no longer foamed. The original juice foamed very heavily and was difficult to manage during testing for polysaccharides.

Table 3. Results of treating beet diffusion juice with cationic polyaluminum preparations. The highlighted rows show the best action within treatment groups.

Treatment	% Removed from Beet Juice by Treatment		
	Color	Turbidity	Polysaccharides
Heat only	33.8	48.1	23.2
Heat + Lime	20.0	65.7	4.6
Heat, Lime, Flocculent (control)	**27.7**	**73.9**	**3.3**
Heat, lime, flocculent + EC 309 HB	50.8	75.7	6.4
Heat, lime, flocculnt + EC 409	42.7	75.0	3.9
Heat, lime, flocculent + EC 462	**56.7**	**92.8**	**18.4**
Heat, lime, flocculent + EC 463	**54.8**	**89.8**	**12.5**
Heat, lime, flocculent + EC 464	**53.9**	**89.1**	**21.6**
Heat + EC 309 HB only (no lime, no floc)	39.3	45.4	24.5
Heat + EC 409 only (no lime, no floc)	44.2	54.6	31.0
Heat + EC 462 only (no lime, no floc)	12.7	57.1	33.5
Heat + EC 463 only (no lime, no floc)	**50.5**	**81.2**	**32.9**
Heat + EC 464 only (no lime, no floc)	37.6	52.4	29.1

As with cane juice, the settling rate of the aluminum-treated beet juices in the lime system was not as good as that for the control juice (heat, lime and flocculent), shown in Figure 13.
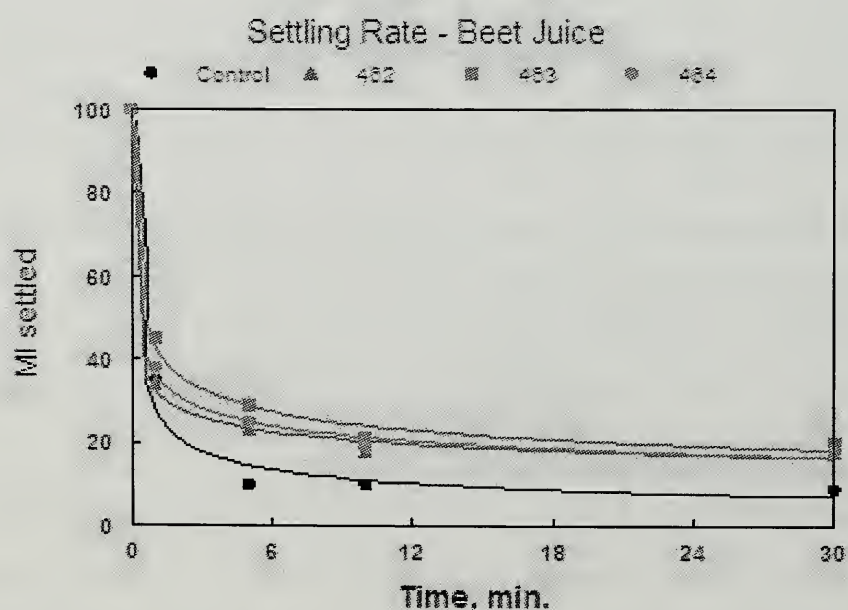
Figure 2. Settling rate of beet diffusion juice treated with lime, flocculents and aluminum coagulants compared to the control.

Sucrose loss. We also determined if sucrose was degraded by the use of aluminum coagulants. Changes in glucose and fructose were monitored in the treated juices. The results are shown in Table 4. Invert sugars did not increase in either cane or beet juice, and purity increased by about 2 points (purity data not shown).

Table 4a. Glucose and fructose in cane juice treated with aluminum coagulants in conjunction with lime, heat and flocculant.

Cane Juice	% Glucose on solids	% Fructose on solids
Mixed juice	1.77	1.63
EC 309 HB	1.59	1.53
EC 409	1.58	1.53
EC 462	1.59	1.49
EC 463	1.59	1.66
EC 464	1.57	1.59

Table 4b. Glucose and fructose in beet juice treated with aluminum coagulants in conjunction with lime, heat and flocculant.

Beet Juice	% Glucose on solids	% Fructose on solids
Diffusion juice	0.94	1.12
Heat, lime & flocculent only	0.59	0.49
EC 309 HB	0.61	0.57
EC 409	0.63	0.86
EC 462	0.57	0.62
EC 463	0.55	1.14
EC 464	0.63	0.50

Mill Study - November and December 2005

A mill experiment was carried out for two days each in November and December 2005, using EC 462 and later EC 461 (not available at the time of the laboratory studies). The EC 461 was chosen because it has a slightly higher content of polyamine (65% vs 50%), which could aid in color removal. Both PAC gave similar results. PAC was added into the clarification tank after liming. The amount added was determined by the concentration of polyquaternaryamine in the sample, based on sugar solids, as regulated by the CFR (Table 1) .

Results are summarized in Table 5. There was improvement in removal of turbidity, color and ash, and an increase in pH, compared to traditional clarification. Polysaccharide concentration was not improved. There was a lot of bagacillo in the juice (conditions were wet and muddy on both occasions), and the heat of clarification may have caused hemicellulose to leach out of the bagacillo. Analysis of treated samples showed that there was no aluminum carry-over into the clarified juice (0.1 to 0.4 ppm).

Table 5. Summary of mill study results

Parameter	Comparison to standard clarified juice
Turbildity	3% improvement
Ash	Decreased 3-10%
pH	Increased by 1.25 units, compared to 0.79 increase after standard clarification
Color	Color removed with standard clarification averaged 16%; color removed with PAC addition was 35%.
Polysaccharides	No improvement. Increase in both treated and regular clarified juice compared to mixed juice.

Conclusions

The exceptional results of the jar tests on cane juice show the potential of PAC for improving the quality of clarified cane juice. The preliminary mill results were very promising.

Addition in the mill needs to be optimized. In retrospect, we felt that the point of addition of the PAC had not been optimized, and that further benefit may have been obtained by adding it at the same time as the lime and flocculent, as was done in the laboratory tests.

The pH increase noted in the mill study may allow reduction in lime usage. The ability of PAC to synergistically enhance the action of lime clarification was shown in the bench studies.

PAC use in beet diffusion juice also showed potential, but further work needs to be done to determine if PAC can be effective in beet sugar processing and to optimize its use.

We also feel that greater benefit would be obtained if the amount allowed to be added could be on the basis of juice and not on the basis of solids. The polyamine quantity is regulated for use in high-solids processes (refineries), and we feel that cane or beet juice could be treated with levels allowed for raw water treatment, e.g., 200-1000 ppm. These are the levels reported used in Brazil and India. The levels used in the bench studies reported here were ~150 ppm on juice, which also explains the higher level of performance compared to the mill performance.

Acknowledgments

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The Content of Polyphenols in Sugars of Different Origins

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Introduction

Increasing production of white cane sugar, particularly with the opening of the European market for cane sugar, makes identification of white sugar origin an important issue. It is therefore important to find sugar components that are characteristic of cane and beet sugar. The content of polyphenolic components seems to be one of such markers. We need an analytical method that is capable of differentiating between beet and cane sugars, and which can also identify mixtures of cane and beet sugars (1).

Sugar cane contains polyphenols – phenolic acids and flavanoids. These compounds have also been found in cane sugar products such as syrup, molasses and brown sugar. The main polyphenols in cane products are phenolic acids, such as vanillic acid, vanillin, p-coumaric acid, ferulic acid, and benzoic acid (Figure 1). Those compounds come mainly from the sugar cane plant as products of degradation of the metabolites from the cane (2-4).
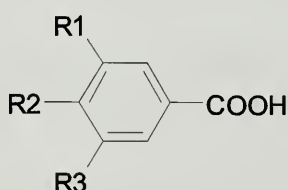
Acid		R1	R2	R3
p-Hydroxybenzoic	4-Hydroxybenzoic	H	OH	H
Protocatechuic	3,4-Dihydroxybenzoic	OH	OH	H
Vanillic	4-Hydroxy-3-methoxybenzoic	OCH_3	OH	H
Syringic	3,5-Dimethoxybenzoic	OCH_3	OH	OCH_3
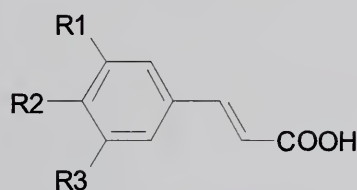
Acid		R1	R2	R3
p-Coumaric	4-Hydroxycinnamic	H	OH	H
Caffeic	3,4-Dihydroxycinnamic	OH	OH	H
Ferulic	4-Hydroxy-3-methoxycinnamic	OCH_3	OH	H
Sinapic	4-Hydroxy-3,5-dimethoxycinnamic	OCH_3	OH	OCH_3

Figure 1. The main polyphenols reported in cane products and cane sugar.

Materials and Methods

The investigated materials consisted of:

> 4 samples of white beet sugar (A,B, C, D)
> 3 samples of brown cane sugar (E, F, G)
> 1 sample of white cane sugar (H)

The total content of polyphenols in sugar samples was determined by means of the Folin Ciocalteau method after extraction (4). Polyphenols were extracted from the sugar samples by means of an SPE (solid phase extraction) technique. For more precise characterization of polyphenolic compounds in the analyzed sugars, the extracts were analyzed by HPLC with UV DAD detection.

Isolation of Polyphenols

Phenolic compounds were separated from sugar on SPE "Stata X" columns. After extraction of phenolic compounds on the SPE column, the retained phenols were eluted by alcohol.

Determination of Polyphenols

The total content of polyphenols was determined by means of the Folin Ciocalteau spectrophotometric method on a spectrophotometer (HITACHI U-1800).

The content of phenolic compounds was determined by means of HPLC on a Dionex chromatographic system with Synergi 4u Fushion RP 80 A 150×2 mm column and UV-VIS UVD 340U detector. Detection was performed at 280 nm and the absorption spectra of compounds were recorded between 210 and 350 nm.

Two elution solvents were used:

> A - 0.05% aqueous phosphoric acid
>
> B - 0.05% phosphoric acid in acetonitrile

The gradient program was: 0 min 4% B; 33 min 50% B; 34 min 50% B; 35 min 4% B.

Results

The total phenolic content of all analyzed sugars ranged from 0.2 to 26.3 mg/kg (Figure 2). In beet white sugars (samples A, B, C, D) the content of polyphenols was very low - below 0.5 mg/kg. The white cane sugar (sample H) contained significantly more polyphenols - 3.5 mg/kg. The highest content of total phenolic compounds was noted in brown cane sugars (samples E, F, G), on average 23.5 mg/kg. These results show that the polyphenols are specific components of cane sugars. The high content in brown sugars is caused by mother syrup and is correlated with sugar color, because phenolic compounds are strongly involved in the color formation of sugar products (5).

For more precise characterization of polyphenolic compounds in the analyzed sugars the extracts were analyzed by HPLC with UV DAD detection. The sample profiles of white beet sugar, white cane sugar and brown cane sugar are presented in Figure 3.
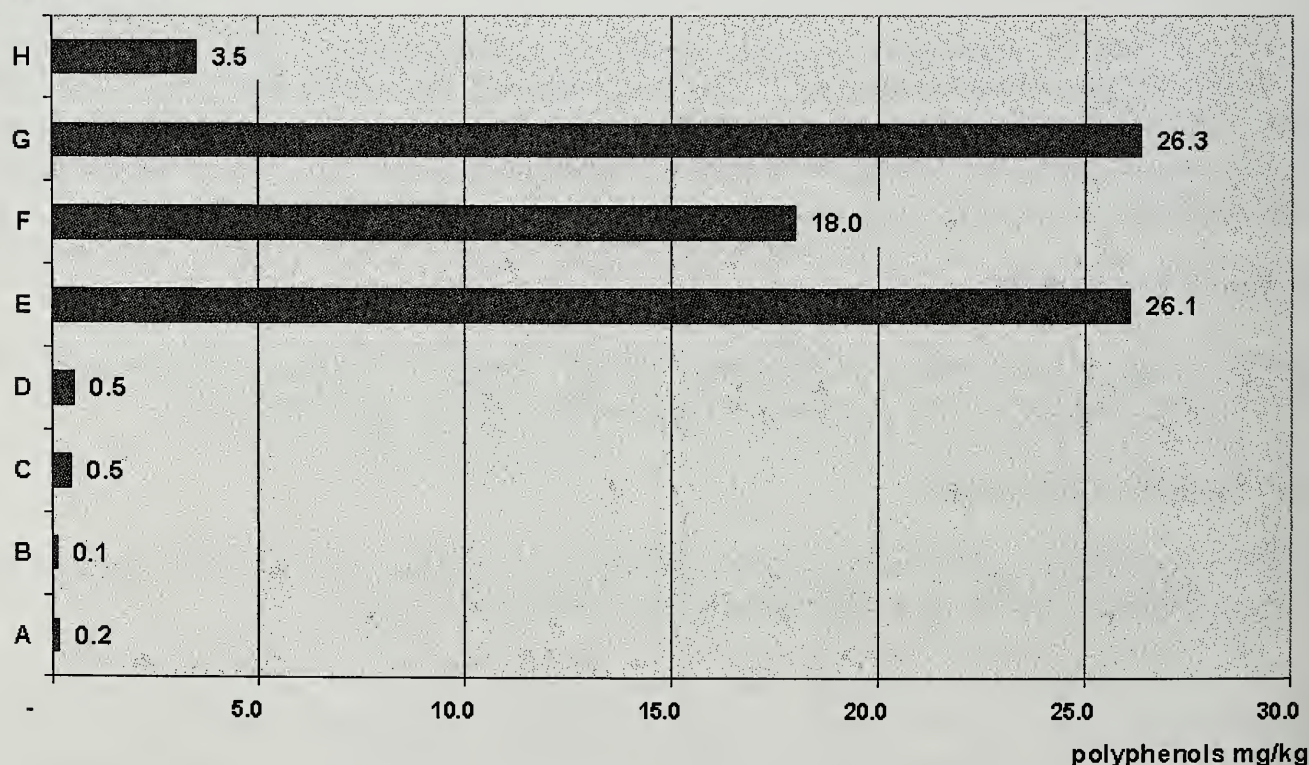
Figure 2. The content of total polyphenols in beet (A, B, C, D) and cane (E, F, G, H) sugars.
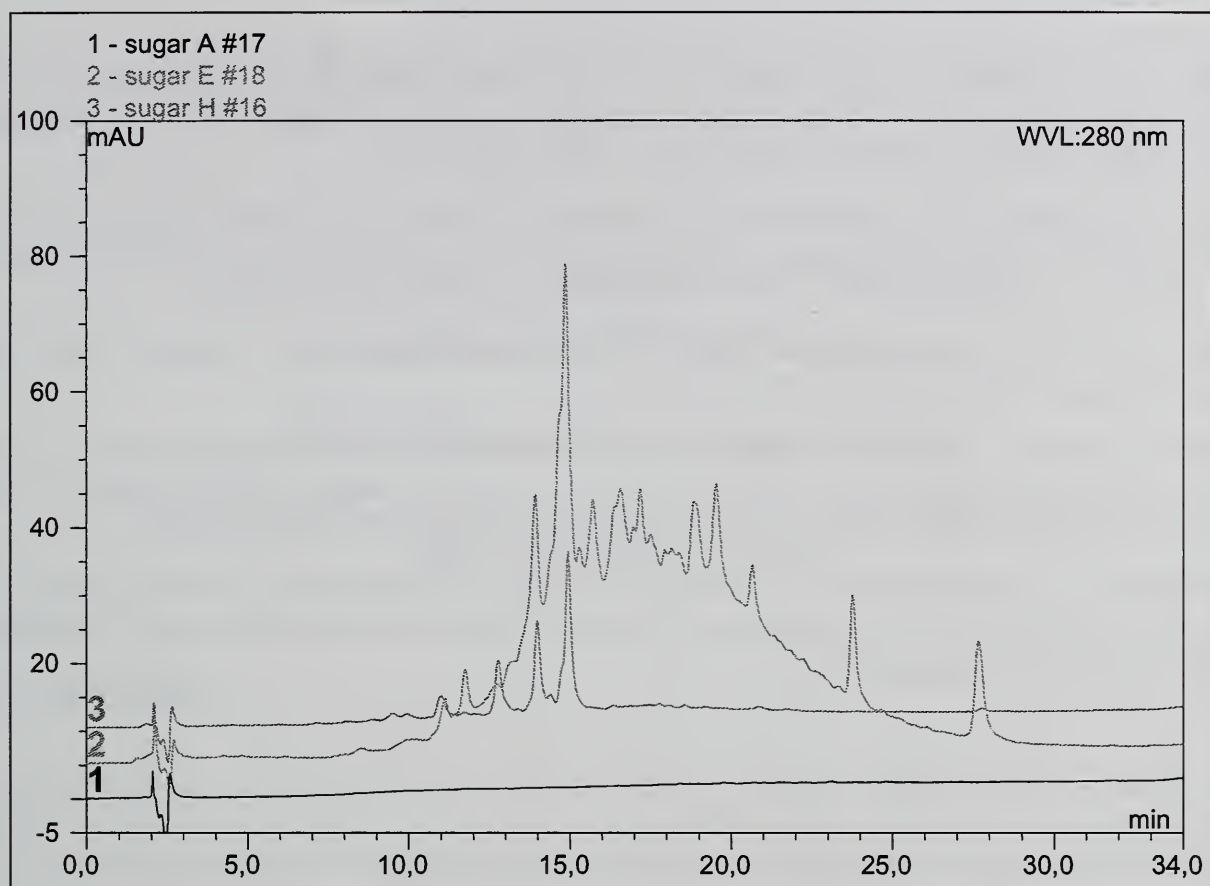
Figure 3. HPLC profiles of white beet sugar (1), brown cane sugar (2), and white cane sugar (3).

The specific peaks for cane sugars were noted. At this investigation stage we didn't identify any phenolics compounds but we found only the characteristics peaks for this group of components. We expect that the main polyphenols detected in the analyzed cane sugars were syringic acid, vanillic acid, and other benzoic acid and cinnamic acid derivatives. We found that there are no such peaks in beet sugars, and it seems to us that peaks characteristic of polyphenolics compounds in cane sugars can be used as a marker for identification of sugar origin.

Conclusion

The content of polyphenols determined by the Folin Ciocalteau method in the cane sugars tested ranged from 3.5 mg/kg for white cane sugar to 26.3 mg/kg for brown sugars. For beet sugars the content of polyphenols was much smaller, ranging from 0.1 mg/kg to 0.5 mg/kg.

HPLC analysis showed significant differences between the analyzed sugars in the content of polyphenols and their compounds. HPLC profiles can be used as markers of sugar origin for differentiating cane sugar from beet sugar. Study should be continued on identification of phenolic components in cane sugars and for the identification of mixtures of cane and beet sugars.

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The Content of Soluble and Insoluble
Calcium in White Sugar

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Introduction

Nowadays many industrial sugar consumers, as a result of high competition in the sugar market, introduce additional requirements for white sugar quality. These requirements concern mainly the content of various impurities in sugar and their impact on sugar and sugar solution properties, such as filtration time, the content of insoluble matter, creating flocks, turbidity or foaming (1-3).

One of the most important properties of sugar is the turbidity of the sugar solution. Turbidity of sugar solutions is usually defined as the difference between the absorption before and after filtration of 50 % sugar solution (3). The main causes of turbidity are insoluble calcium salts such as carbonates, oxalates, phosphates and unfiltered sediments of thick juice and remelt syrups. Turbidity of sugar solutions depends on the quality of beetroot, extraction conditions, the amount of calcium used in juice liming, alkalinity of carbonation, quality of filtration of thick juice and remelt syrup (2, 4).

As reported in the literature (2, 4) turbidity of aqueous sugar solutions is correlated with the content of conductivity ash and the content of insoluble matter. It is generally assumed that calcium carbonate is one of the main impurities in sugar responsible for the turbidity as well as for the content of insoluble matter and conductivity ash (4 – 6).

This paper presents the content of soluble and insoluble calcium in white sugars from different Polish sugar factories. The calcium content in sugar was determined by means of atomic absorption spectrometry (FAAS) both in soluble form and in two insoluble forms retained on membrane filters with pore size 8.0 μm and 0.45 μm.

Materials and Methods

The materials for this study were nine white sugar samples from different Polish sugar factories. In those samples were determined:

- the content of conductivity ash by GS2/3-17 ICUMSA Method (7)

- the content of water insoluble matter by GS2/3-19 ICUMSA Method (7)

- the turbidity of sugar solution as difference in the results of absorbance measurements before and after filtration of the 50 % sugar solution (3)

- the content of Ca by means of atomic absorption spectrometry (SOLAAR 969) (8).

The content of calcium was determined both in water soluble form and in two water insoluble forms. The aqueous sugar solution was filtered first on membrane filter 8.0 μm and after this filtration was filtered on membrane 0.45 μm. The first insoluble fraction of Ca contained particles of calcium with diameter more than 8.0 μm, and the second fraction contained particles with diameter from 0.45 μm to 8.0 μm.
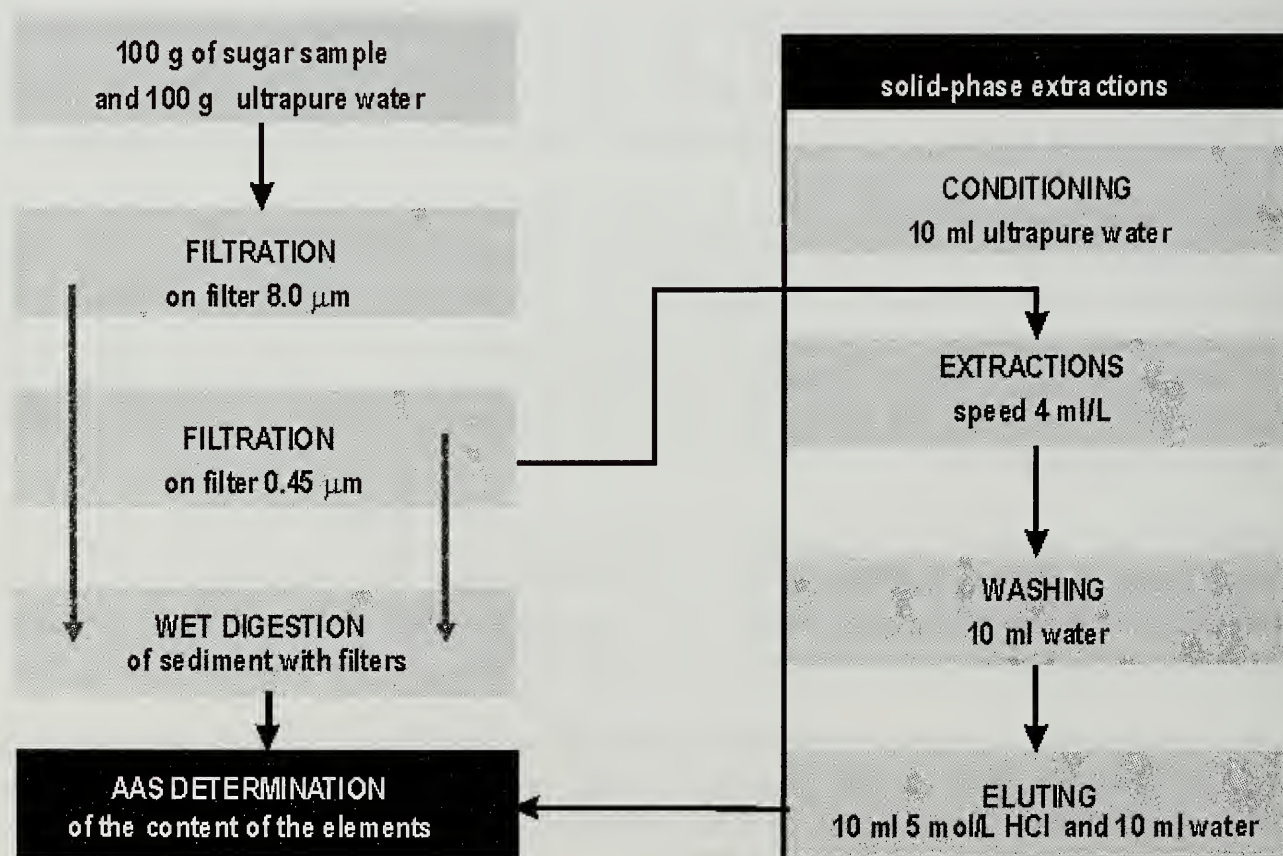
Figure 1. Scheme of sugar samples preparation for determining the content of three forms of calcium.

Results and Discussion

The analyzed sugar samples contained from 0.0069 % to 0.0170 % of conductivity ash, on average 0.0106 % (Figure 2). The average content of insoluble matter was 7.3 mg/kg for sugar samples, and ranged from 2.0 mg/kg to 13.7 mg/kg (Figure 3). The turbidity of analyzed sugars was from 4.2 IU to 136.5 IU, with the average turbidity being 42.4 IU (Figure 4).
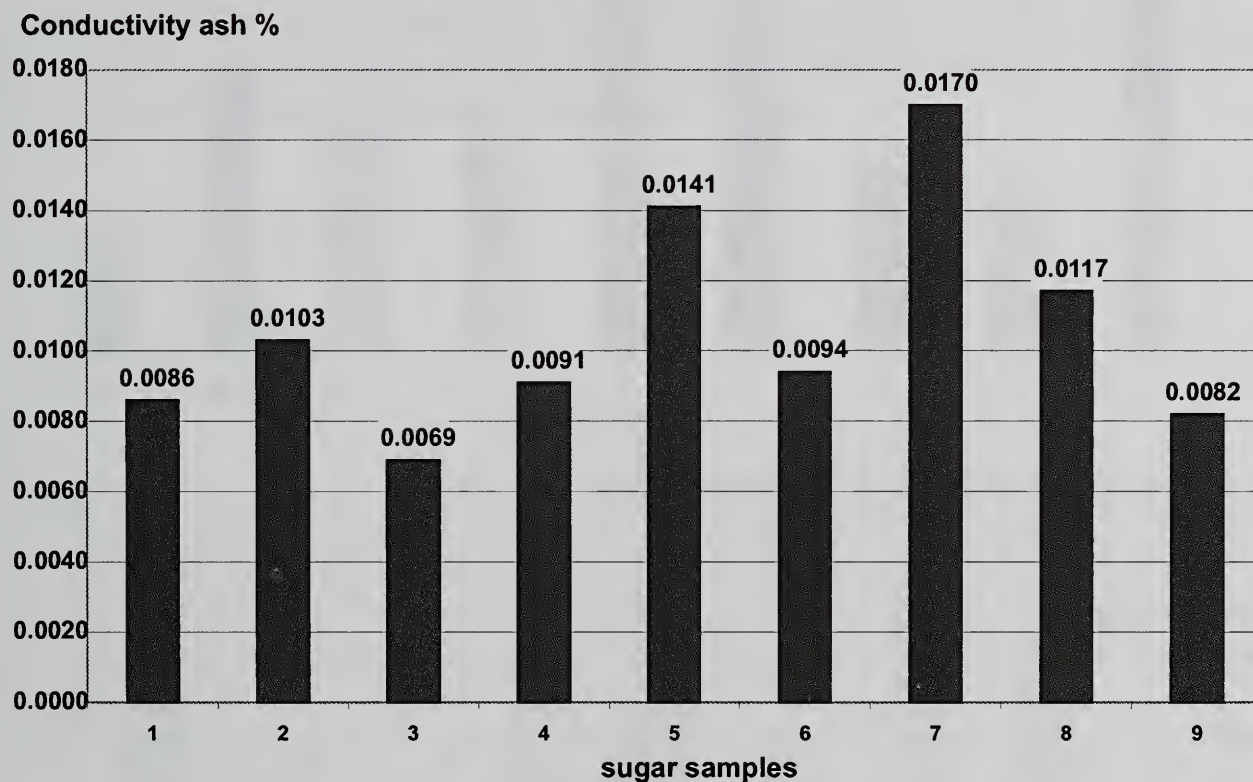
Conductivity ash %

Figure 2. The content of conductivity ash in sugar samples.

Insoluble matter mg/kg
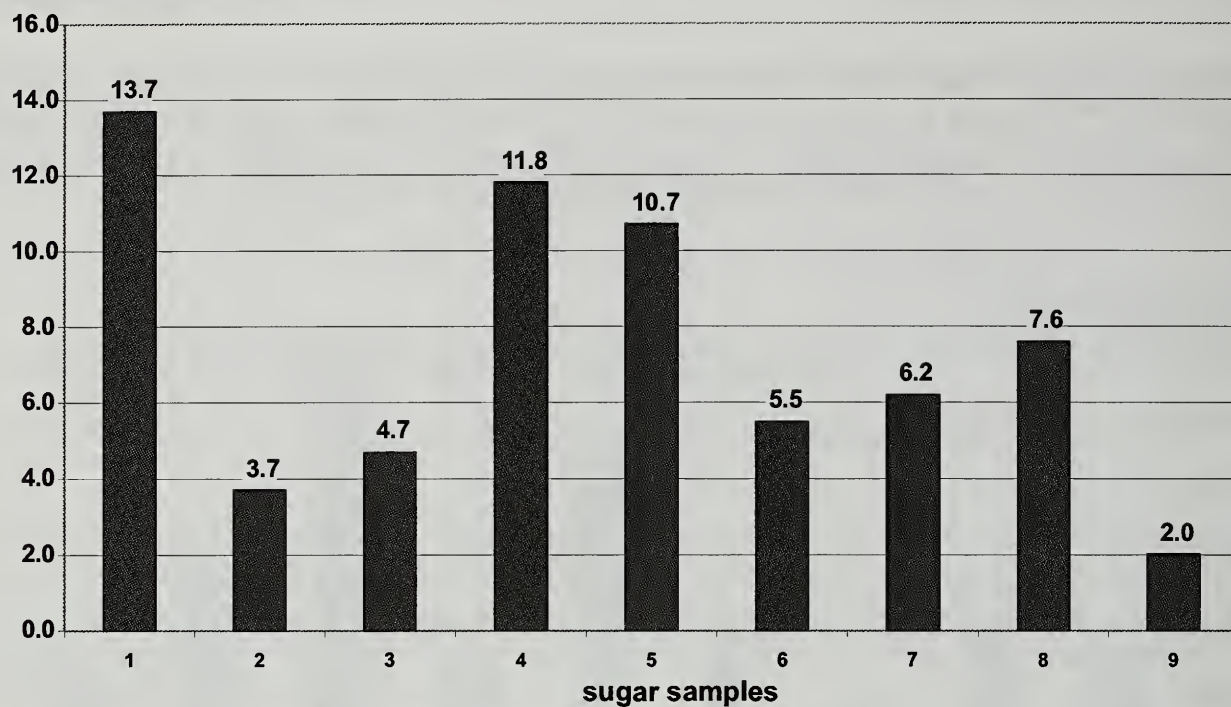
Figure 3. The content of the insoluble matter in sugar samples.

Turbidity IU420
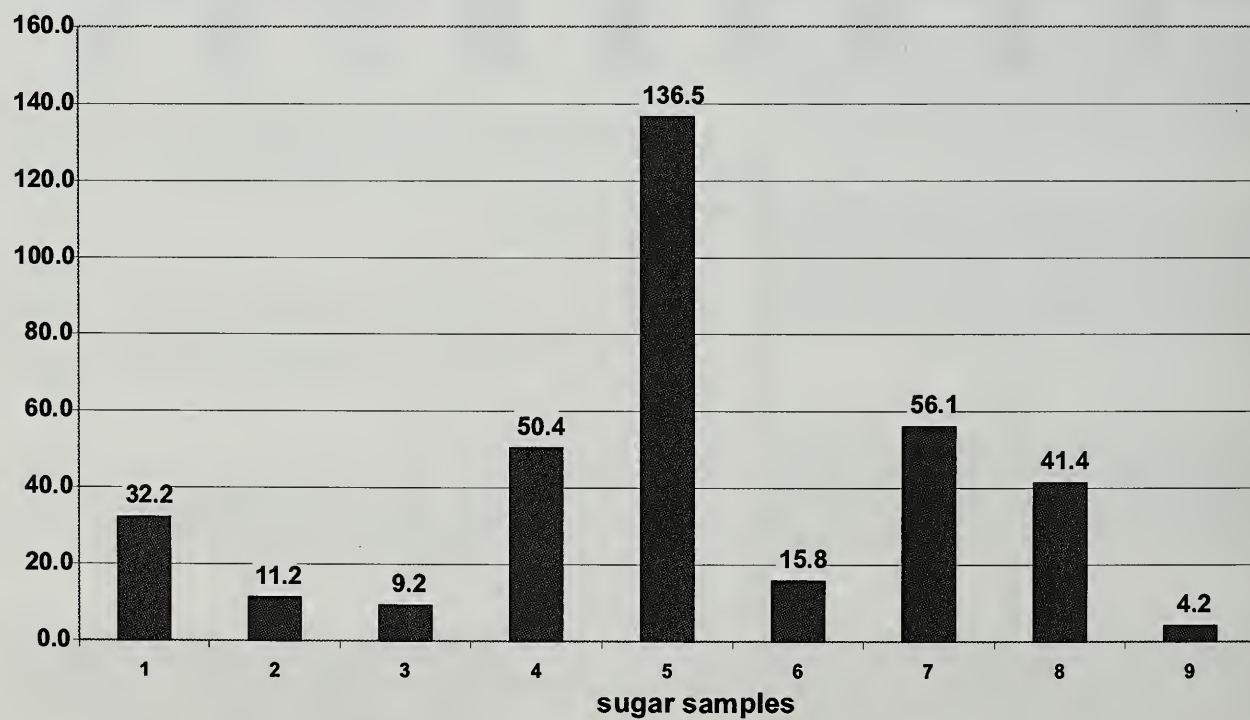
Figure 4. The turbidity of sugar solutions.

Total content of calcium in the analyzed sugars was significantly different and ranged from 2.5 mg/kg to 40.6 mg/kg, on average 11.6 mg/kg. The calcium in analyzed sugars was mainly in soluble form. The proportion of soluble calcium to total calcium in the sugars was different, and ranged from 71% to 95%, on average 84%. The proportion of two insoluble forms of calcium in insoluble calcium is shown in Figure 5. The proportion of these forms was very different for different sugar samples. In sugar sample 5 only 24% of insoluble calcium was retained on an 8.0 μm filter, but in sugar sample 7 this fraction contained 83% of insoluble calcium (Figure 6). The content of calcium in the first fraction was generally lower than the content of calcium in the second fraction. The highest content of calcium in the first fraction was noted in sugar sample 5 with the highest turbidity. The main element of insoluble matter in sugars was calcium whose content in insoluble matter was from 0.2 mg/kg to 1.7 mg/kg.
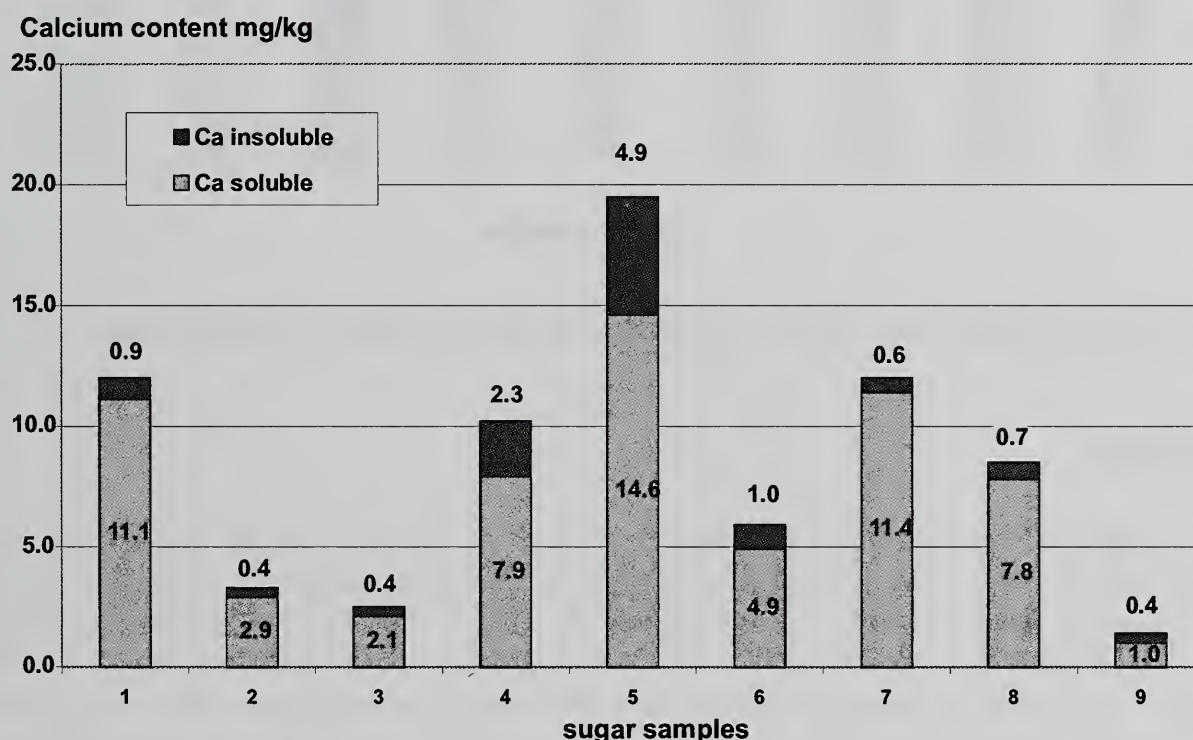
Figure 5. The content of soluble and insoluble calcium in sugar samples.
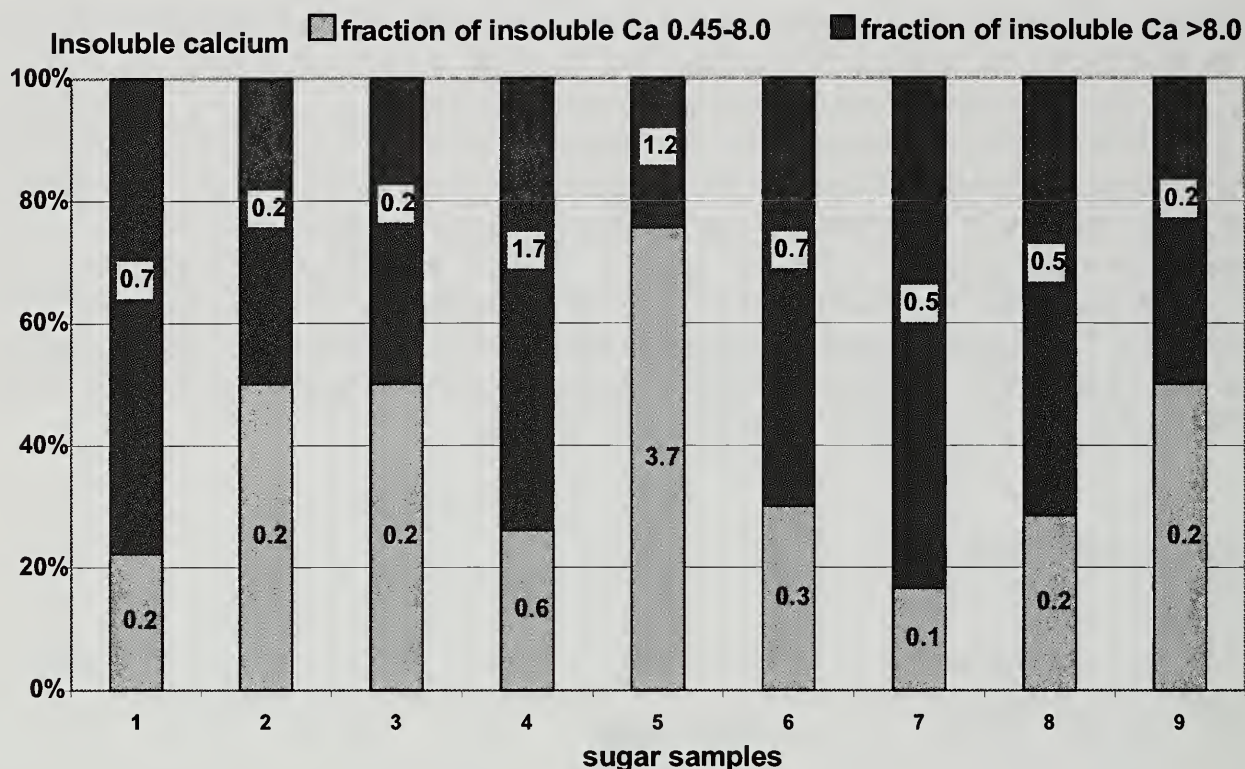
Figure 6. The proportion of two forms of insoluble calcium in total insoluble calcium.

Conclusion

This study confirms the correlation between the content of conductivity ash and the content of calcium in soluble form. The content of insoluble calcium in general was correlated with the value of insoluble matter in the analyzed sugars (R = 0.91). We found significant correlation between the turbidity and the content of calcium, especially of the total content of insoluble calcium (R = 0.91) and the content of calcium in the fraction of insoluble matter with particles from 0.45 μm to 8.0 μm (R = 0.90). We also found a weaker correlation between turbidity and the content of soluble calcium (R = 0.86).

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Measurement of α-Amino Nitrogen in Sugar Using Fluorescence Spectroscopy

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Abstract

Protein is considered one of the necessary elements for floc formation in refined cane sugar, but because of the very low levels of protein in white sugar, it is difficult to measure. We recently examined o-phthaldialdehyde (OPA) as a sensitive reagent to measure α-amino nitrogen in white sugar. OPA is a fluorescent tag that reacts with primary amines to help identify proteins. Glutamine and asparagine, the most common amino acids in cane juice, were used to construct calibration curves and their combined curve used to measure low levels of primary amino nitrogen in refined cane sugar, white beet sugar, and Indian plantation white sugar. Small volumes of sugar solution were reacted in a 96-well plate and read in an automated fluorometer, using excitation at 340 nm and emission at 465 nm. Only a fraction of a milliliter is needed for the test, and reaction is immediate. The limit of detection in sugar solutions was about 0.1 ppm.

Introduction

Acid beverage floc (ABF) is a severe quality issue for beverage manufactures using refined cane sugar. Research has been conducted by SPRI and numerous others for many years investigating the acid beverage floc problem in cane sugar (1). Floc formation in cane sugar solutions is due to the interaction of negatively charged polysaccharides, specifically the indigenous sugarcane polysaccharide (ISP), and positively charged proteins present in sugar solutions (2). The most commonly found amino acids in cane juice are glutamine and asparagine.

Several floc tests are used around the world today to predict the floc potential of white cane sugar. Traditionally these tests require making high or low brix solution, acidifying the solution to mimic acid beverage conditions and observing the solution for ten days. Three tests that are commonly used are the Coca-Cola/ICUMSA test, the Pepsi Cola test, and the Australian test. The difference between the Coca-Cola and Pepsi Cola test is that the Pepsi Cola test heats the acidified solution just to boiling before allowing it to sit for 10 days. The Australian test uses a lower brix solution with a preservative added. No successful rapid floc predictor test has been developed to date.

Several protein binding dyes have been studied in order to develop a rapid test for floccing potential in white cane sugar (3). Most of these dyes absorb and emit light in the visible range. In this research, we examined a fluorescent dye, o-phthaldialdehyde (OPA), in an attempt to quantitate the amount of α-amino nitrogen present in white cane sugar in hopes of correlating the amount of α-amino nitrogen present to the floccing potential of the sugar. Fluorescent dyes are very sensitive, allowing a lower limit of detection for the species being analyzed (4,5). OPA mixed with 2-mercaptoethanol gives a bright blue fluorescence with a short reaction time and is easily adaptable to a 96-well fluorescence plate reader allowing for many samples to be analyzed at once. The limit of detection for protein using OPA reagent is 0.1 ppm.

Experimental

Cane white sugars (floccing and non-floccing) and white beet sugars were obtained from various SPRI sponsor companies. Plantation white sugars from India were also obtained. Glutamine and asparagine were purchased from Sigma Chemical Company and dissolved in deionized water in concentrations of 0.001 ppm, 0.01 ppm, 0.1 ppm, 0.5 ppm, 1 ppm, 5 ppm, and 10 ppm to construct a standard calibration curve. The OPA reagent was purchased from Sigma Chemical Company. The concentration of OPA in this reagent is 1mg/ml stabilized with 2-mercaptoethanol.

The standard curve was created by treating 10 ml of amino acid sample, in the concentrations listed above, with 1 ml of OPA reagent and allowing it to react for 10 minutes. For each sample, 200 μl was pipetted into one well of a 96-well plate. The plate was read using a Perkin Elmer HTS 7000 BioAssay Reader with an excitation wavelength of 340 nm and emission wavelength of 460 nm. Each sample was blanked against an unreacted amino acid solution of the same concentration. This corrected for fluorescence due to the plate.

Sugars tested included: Refined white cane sugar with a positive floc test (floccing sugar) , refined white cane sugar with a negative floc test (non-floccing sugar), plantation white sugar (PWS), raw cane sugar, and refined white beet sugar. The sugar solutions were prepared to 50 Bx and 10 ml of that solution was reacted with 1ml of OPA reagent. The reagents were mixed thoroughly and reacted for 10 minutes. For each sample, 200 μl was pipetted into one well of a 96-well plate. The plate was read using a Perkin Elmer HTS 7000 Bio Assay Reader with an excitation wavelength of 340 nm and emission wavelength of 460 nm. Each sample was blanked against the unreacted sugar solution. This corrected for the fluorescence due to the plate and to the sugar colorants. The fluorescence was read within 20 minutes of reaction.

Results and Discussion

The most commonly found amino acids in sugarcane juice, glutamine and asparagine, were used to construct a standard curve to estimate the amount of α-amino nitrogen (proteins or amino acids) found in sugar samples. This standard curve is shown in Figure 1. The linear regression line from the average of these two amino acid curves was used in the calculations for the α-amino nitrogen for the samples tested.

Sugar in solution reacts instantly with the OPA reagent forming a pale blue to bright turquoise fluorescence depending on the concentration of the amino nitrogen present (see Figures 2 and 3). The background fluorescence was about 600-650 which was attributed to the 96-well plate. Cane sugar colorants also have some inherent fluorescence at this wavelength, and therefore an untreated sugar solution was used as a blank for each sugar sample tested. See Table 1 for examples of typical fluorescence values for treated and untreated sugar samples.
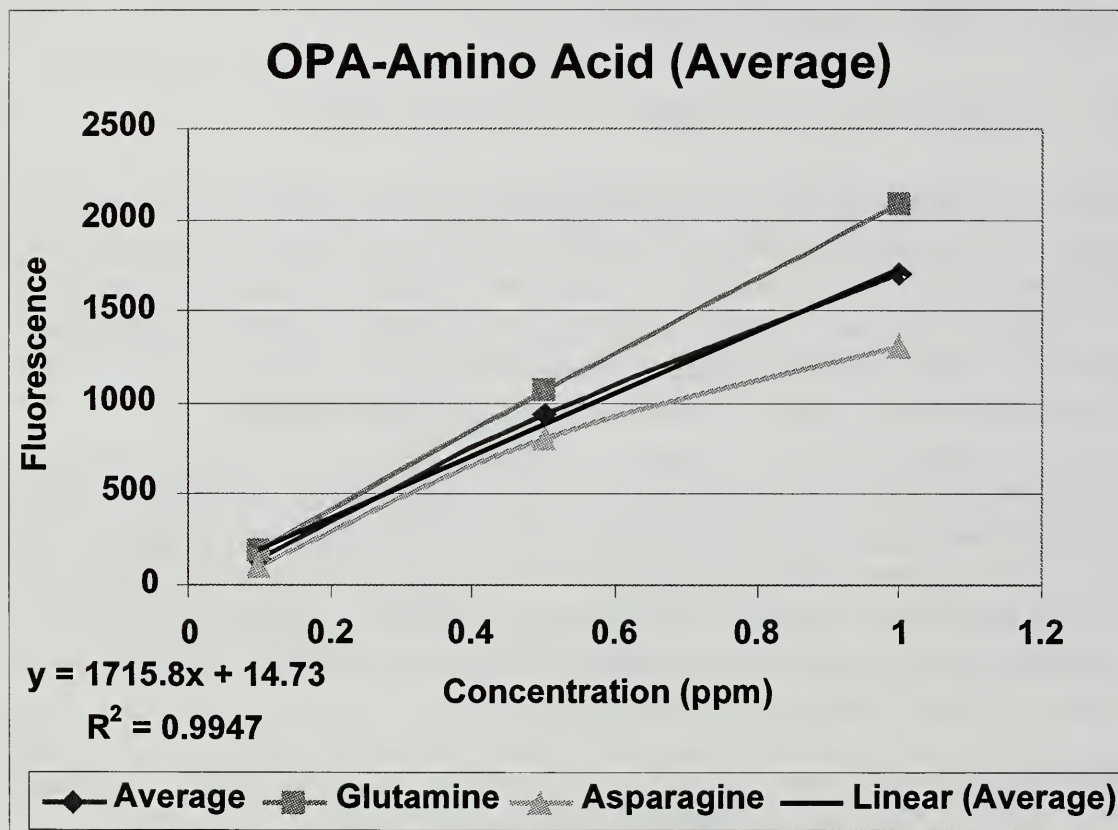
Figure 1. Standard curve for amino acids in cane juice.

Table 1. Fluorescence of OPA-derivatized and unreacted sugar solutions.

Sample	OPA- fluorescence of reacted solutions	Fluorescence of unreacted solutions	Fluorescence due to OPA
Floccing cane sugar	717	602	115
Non-floccing cane sugar	653	686	0
Plantation white cane sugar	8903	650	8253
Raw cane sugar	2298	1075	1223
Water	559	560	0

Figures 2 and 3 illustrate the fluorescence of different sugars showing the range of color the OPA derivatized sugars produce. In Figure 2, PWS derivatized with OPA is shown. The PWS is a low color sugar product similar in color to a white sugar. The plantation sugar has a low intrinsic fluorescence but after derivatization with OPA has a high fluorescence as evident by the bright turquoise color produced indicating high α-amino nitrogen content. Figure 3 shows the fluorescence of two OPA derivatized refined white sugars – one floccing (left) and one non-floccing (right). The fluorescence of the two refined white sugars isn't distinguishable by the human eye, but measuring the fluorescence in the fluorometer gives different values for the α-amino nitrogen content of the two sugars.
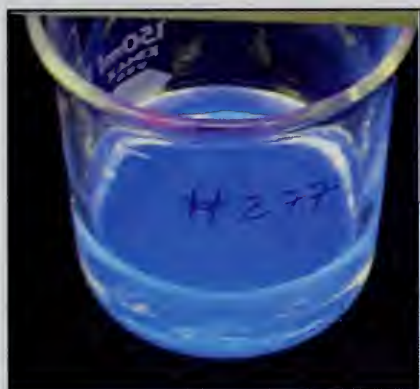
Figure 2. PWS and OPA.

Figure 3. Refined cane sugars and OPA. Left. Floccing; Right: Non floccing.

Table 2. Average α-amino nitrogen in white sugars (ppm).

Sugar Type (n = number of samples tested)	Average α-Amino Nitrogen, ppm
Beet sugar, white (n = 8)	1.7
Cane sugar, refined (n = 19)	0.2
Plantation white sugar (n = 26)	2.4

Table 2 shows the average α-amino nitrogen content of the various types of sugars tested. Beet white sugars had an average α-amino nitrogen content of 1.7 ppm, refined cane sugar (floccing and non-floccing) had an average content of 0.2 ppm, and plantation white had and average of 2.4 ppm α-amino nitrogen. Of the eight white beet sugars tested, the α-amino nitrogen values ranged from 0.0 ppm to 2.7 ppm. The floccing refined cane sugar α-amino nitrogen content ranged from 0.1 to 3.6 ppm, while the non-floccing refined cane sugar values were 0.0 ppm to 1.1 ppm. The plantation white sugar α-amino nitrogen values were much higher at 0.4 ppm to 4.2 ppm.

Conclusion

OPA is a fluorescent tag that reacts strongly and rapidly with primary amines to help identify proteins in various aqueous mixtures. This tag was shown to be a sensitive reagent for determining low levels of α-amino nitrogen in white sugar. The limit of detection for this experimental setup was determined to be 0.1 ppm. The α-amino nitrogen in cane sugar, floccing or non-floccing, proved to be just above that threshold at 0.2 ppm. This test did not clearly distinguish floccing cane sugars from non-floccing cane sugars. This may be because the average α-amino nitrogen content in many cane sugars is very near the limit of detection for this method.

Acknowledgments

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Report of a Collaborative Study on Dextran in White Sugar Using an Antibody Test

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Abstract

The determination of dextran in white sugar is important for the manufacturers of shaped hard candies, in particular, round candies, such as Life Savers. A dextran concentration greater than 125 ppm will cause the sugar crystal to elongate, resulting in distortion of the candy geometry. Thus, a candy that is supposed to be perfectly round will become slightly oblong. Very few refiners test for dextran in the finished refined sugar. A collaborative study, using the accepted IUPAC protocol, was conducted, with ten laboratories participating. Of these ten, only two laboratories routinely tested for dextran in white sugar. The Midland Monoclonal Antibody method (MCA) was chosen for its rapidity and ease. Samples ranged in concentration from 15 to 240 ppm dextran. Statistical analyses of the results showed that the test performed adequately, with outliers and variability ratios falling within acceptable parameters for all but the sample with the lowest concentration.

Introduction

Dextran is a well-recognized problem in sugar processing, and is routinely measured in mills. Methods for measuring dextran include the haze test (ICUMSA Method GS1-15 for dextran in raw sugar) and several variations, including the Roberts copper method (an official AOAC method for determining dextran in raw sugar), and the Midland monoclonal antibody method (MCA). Each method measures a different aspect of dextran, based on molecular weight and specificity. The haze method, which utilizes 50 percent ethanol for the initial precipitation of dextran, measures high molecular weight dextran and is non-specific for dextran. The Roberts method utilizes 80 percent ethanol for the initial precipitation of dextran and uses copper to speciate dextran. Roberts picks up a wider MW range of dextran on the lower end because of the precipitation with more concentrated ethanol (80%), and thus gives higher results. The MCA method uses an antibody specifically tailored to react with dextran. Comparative tests have shown that MCA probably favors high MW dextran and gives results similar to the haze method.[1]

Dextran is seldom measured in white sugar, as the levels are low and it does not usually cause a problem in products. The one exception, however, is the effect of dextran on the shape of hard candies. Kraft Foods demonstrated that levels of 125 ppm and higher of dextran in refined sugar will cause significant change in candy geometry, and set this level as a specification for white sugar.[2] The effect of dextran in candy manufacture has long been recognized. [3,4]

An earlier screening of 41 refined cane, beet and plantation white sugars indicated that the three dextran methods, haze, MCA and Roberts, gave comparable results.[5] This indicated that the dextran in white sugar is very high MW.

The makers of hard candies need a validated test to measure dextran in white sugar, and the antibody test kit sold by Midland Research Laboratories (Ecolabs), already in use by Kraft, was chosen as the method for collaborative testing, due to its rapidity and ease of use.

Organization of the Test

The method used for determining dextran by the antibody method was adapted for white sugar from the method used by Midland for cane juice and is shown in Appendix 2.

The ICUMSA/IUPAC protocol for collaborative tests was followed. Each participant was mailed a set of instructions, a response sheet, a sheet for comments, 12 packets of sugar, and two practice samples with the known values provided. The 12 sugar samples represented 6 sugars sent out as blind duplicates Participants were asked to analyze each sample one time only. The participants provided all the rest of the test materials.

The statistical analyses of the results was done using the Excel software package from the Association of Official Analytical Chemists (AOAC) for determining the statistics of a collaborative study using the IUPAC protocol. In the results that are given, each set of results is identified as a laboratory, even though they may actually represent the same laboratory but different tests or different analysts.

The test is calculated using a one-point conversion factor (CF), but two of the labs calculated results using a dextran calibration curve. Ten labs conducted the MCA test using the CF. Three labs did the haze test. After the instructions and samples were sent out, an error was noted, and participants were notified. Lab 11 did not receive the correction notice in time, so a correction was applied to the Lab 11 CF.

Participants
Ten laboratories participated. The list below includes which test each laboratory conducted. When reporting the results, each of the methods is identified as a "Lab" even though it may represent the same company.

> Imperial Sugar Company, Colonial, Gramercy, Louisiana – MCA with CF
> Imperial Sugar Company, Savannah, Georgia – MCA with CF
> Kakira Sugars, Uganda – MCA with CF

     Kraft Foods – MCA with CF and MCA with a calibration curve
     Lantic Sugar, Montreal, Canada – MCA with a calibration curve
     RAR, Porto, Portugal – MCA with CF
     Rogers Sugar, Vancouver, Canada – Haze
     SIRI, Jamaica – MCA with CF
     SPRI, New Orleans – two analysts, MCA with CF and Haze
     The American Sugar Refining Company, Chalmette, Louisiana – MCA with CF and Haze

Results

Table 1 shows the results of the practice samples. Table 2 shows the raw data of the test, along with outliers. Table 3 shows the summary statistics of the test.

Table 1. Results of dextran in practice samples and reported CF.

Lab/Method/Analyst	Practice A	Practice B
Given results	46^1, 49^2, 20^3	186^1, 147^2, 130^3
Lab 1, MCA using a calibration curve	17	131
Lab 2, MCA using a calibration curve	21	196
Lab 3, MCA, CF = 2.16	21	167
Lab 4, Analyst 1, MCA, CF = 2.77	12	194
Lab 5, Analyst 2, MCA, CF = 2.92	14	-*
Lab 6, MCA, CF = 2.36	25	204
Lab 7, MCA, CF = 2.37	18	183
Lab 8, MCA, CF = 2.20	20	153
Lab 9, MCA, CF = 2.33	12	170
Lab 10, MCA, CF = 3.91	23	204
Lab 11, MCA, CF = 9.80	31	417
Lab 12, Analyst 1, Haze	39	129
Lab 13, Analyst 2, Haze	19	109
Lab 14, Haze	27	107
Lab 15, Haze	<18**	153

[1] Determined by Roberts Copper dextran method
[2] Determined by SPRI modified haze test
[3] MCA method by an outside Laboratory
* Ran out of antibody   ** Limit of detection   CF = Conversion Factor

Table 2. Results of dextran test in white sugar - raw data.

Lab	Sample A		Sample B		Sample C		Sample D		Sample E		Sample F	
	326	**229**	**657**	**278**	**482**	**765**	**988**	**863**	**141**	**511**	**573**	**381**
Lab 1, MCA calib curve*	16	18	81	64	73	71	76	81	85	77	163	170
Lab 2, MCA calib curve*	46	32	109	109	118	120	117	131	122	138	243	249
Lab 3, MCA	16	12	93	80	91	93	104	100	97	100	222	226
Lab 4, MCA	12	15	96	92	106	103	114	113	115	117	245	267
Lab 5, MCA	12	15	102	101	120	115	118	125	130	128	276	280
Lab 6, MCA	1	11	98	103	104	108	113	134	101	118	267	233
Lab 7, MCA	11	11	80	91	94	100	60	103	113	99	245	238
Lab 8, MCA	21	22	161	100	113	121	122	112	110	156	266	250
Lab 9, MCA	18	12	82	88	88	82	112	117	112	117	235	234
Lab 10, MCA	25	19	123	101	132	122	131	122	134	139	279	276
Lab 11, MCA	52	32	306	321	310	374	460	395	727	433	804	769
Lab 11, rationalized **	14	9	82	86	83	100	123	106	195	116	216	206
Lab 12, Haze	39	44	71	66	79	76	76	89	79	81	191	189
Lab 13, Haze	29	34	89	69	84	79	69	79	69	79	189	184
Lab 14, Haze	10	0	50	50	95	60	60	50	60	50	170	170
Lab 15, Haze	<18	<18	58	68	58	58	63	68	73	68	183	168

* These labs used an antibody calibration curve instead of a conversion factor (CF); see text.
** Refer to discussion for an explanation
Lined cells = Grubbs outliers; Grey cells = Cochran outliers

Table 3. Statistical results of collaborative study on dextran by the MCA method with Lab 11 rationalized results included.

Sample	A (326 & 229)	B (657 & 278)	C (482 & 765)	D (988 & 863)	E (141 & 511)	F (573 & 381)
No. Labs	11	11	11	11	11	11
Labs retained	10	10	11	9	9	11
Lab removed	#2	#8	none	#1 and #7	#8 and #11	none
Reason	Grubbs high	Cochran	--	Grubbs low	Cochran	
Mean	15	93	103	117	113	240
s(r)	3.4	7.6	5.2	8.2	7.0	9.9
s(R)	5.4	13.6	17.2	9.5	18.0	32.5
RSD(r) (%)	23.69	8.17	5.03	6.95	6.14	4.14
RSD(R) (%)	37.11	14.64	16.76	8.05	15.88	13.54
s(r)/s(R)	0.63	0.56	0.30	0.86	0.39	0.30
r	9.6	21.3	14.5	22.8	19.5	27.8
R	15.1	38.1	48.1	26.5	50.4	91.1
Horrat	3.47	1.81	2.10	1.03	2.02	1.93

Discussion

Practice Samples

The practice sample results are shown in Table 1. Lab 11 results and haze results were not used in determining the mean of the practice samples. Statistics showed that the haze results were not the same as the MCA values – slightly higher at the low range of dextran and slightly lower at the higher range of dextran, when compared to MCA results. The number of labs using the haze methods was too small for a thorough evaluation, but they did fall in a range similar to the MCA method.

The CV of the practice samples should correspond to the RSD(R) of the test, and this was indeed the case: Practice Sample A, with CV = 25.1% compared well to the RSD(R) of 23.7% for test sample A, which had a similar dextran concentration. Practice Sample B, at CV = 14.0% compares well to RSD(R) of 15.9% and 13.5% for test samples E and F, respectively.

Practice sample means for MCA results, excluding Lab 11 and haze results:

Sample A = 18 ± 4.52   (C.V. = 25.1%)   Range = 12 - 25   Expected ~ 20 ppm
Sample B = 178 ± 24.94   (C.V. = 14.0%)   Range = 131 - 204   Expected ~154 ppm

Employing a Correction Factor for Lab 11 Results

The original instructions sent to the participants used 10μl of standard 100 ppm dextran solution to determine the conversion factor, and 50μl of sample for the dextran determination. Kim Burns, of Savannah Sugar/Imperial, did a series of evaluations of this instruction, using different combinations of volumes and found that using 50μl of the standard dextran solution gave values more in line with the practice sample values. We sent out a correction to the participants. Lab 11 did not receive the notice in time and had already performed the tests.

We noticed that the conversion factors (CF) calculated by the other labs fell within a close range, as shown in Table 1, except for Lab 11, with CF = 9.80. We averaged all the other CF values to come up with a correction factor to rationalize the Lab 11 results. The mean CF of the eight laboratories shown in Table 1 was CF = 2.63. The correction factor was then calculated as the ratio of this CF to the CF of Lab 11: CF = 9.80/2.63 = 3.73. The correction factor of 3.73 was then applied to all of Lab 11 results, by dividing all Lab 11 results by 3.73. Both uncorrected and corrected results for Lab 11 are shown in Table 2.

We then calculated the statistics according to the AOAC/IUPAC protocol with and without the corrected/rationalized Lab 11 results and found that there was no significant difference in the results. Including or excluding Lab 11 did not change the results in any material way – they remained essentially the same.

Statistical Results of the Test

The statistics of the collaborative test are shown in Table 3. The samples ranged in dextran concentration from 15 ppm to 240 ppm. It is evident from the results of the relative standard deviation of the repeatability (RSD(r)%), that the lowest level of dextran (Sample A) produced much more variation within the lab results (23.69%) than higher dextran results (range 4.14% to 8.17%). This is an indication that the test is at its limit of application at around 15 ppm dextran. Unfortunately, we did not have sugars in the test with dextran values between 20-80 ppm.

Outliers (labs whose values were excluded) did not exceed 2 in any sample. This falls within IUPAC guideline that no more than 2 out of 9 labs be excluded in a collaborative study. Outliers were spread among the labs, as well as spread among Cochran and Grubbs types, indicating there was no particular bias in outlier type.

The Horrat values, except for Sample A, were generally in an acceptable range. Three of the samples did not exceed the 2.00 limit, and two were slightly above, at 2.10 and 2.02. The Horrat value is a ratio that permits evaluating the RSD(R) of a test (see Appendix 1).

The ratio [s(r)/s(R)], is the ratio of the standard deviation of the repeatability and the standard deviation of the reproducibility; this ranged from 0.30 to 0.86. This ratio indicates certain biases in the test. According to Horwitz, this ratio should range from 0.50 to 0.67 (Samples A, B).[6] The average ratio for this study was 0.51. Ratios below 0.50 (Samples C, E, F) indicate the method depends a lot on the individual skill and interpretation of the analyst. Ratios much higher than 0.67 (Sample D) may indicate there is some trouble in individual analyst replications. Greater familiarity with the method can be expected to improve this ratio.

Final Observations

A note should be made of the method used by Labs 1 and 2. Both of these laboratories utilized a dextran calibration curve rather than conversion factors to determine dextran, feeling that this method is more accurate and required less antibody. One of these labs had higher average results (Lab 2) and the other had lower average results (Lab 1) overall, so there appears to be no advantage to using a calibration curve for this test from the point of results, but it will save money on consumables.

It should also be mentioned that except for Labs 1 and 2, none of the other participants measure dextran in white sugar. However, because of increased interest in this parameter in white sugar and a specific request that this test be done, and the willingness of the participants, the test was carried out.

It is concluded that the results of this collaborative study fall within reasonable ranges for acceptability of a validated method. The main purpose to measure dextran in white sugar is to determine if it exceeds the critical concentration of 125 ppm.

Acknowledgments

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Appendix 1. Definition of Terms

CF = conversion factor used in antibody test to determine conversion of NTU to ppm dextran
MCA = monoclonal antibody
CV = coefficient of variation; percent the standard deviation represents of the mean
s(r) = standard deviation of the repeatability (from Table 3).
s(R) = standard deviation of the reproducibility (from Table 3).
RSD(r) = relative standard deviation of the repeatability, reported as a percent.
RSD(R) = relative standard deviation of the reproducibility; reported as a percent.
r = repeatability value = 2.8 x s(r). The amount by which two separate determinations on the same sample by an analyst withing a lab should agree 95% of the time.
R = Reproducibility value = 2.8 x s(R). The amount by which two separate determinations conducted in different labs on identical materials should agree 95% of the time.
Horrat value = Horwitz ratio; a ratio that evaluates the calculated test RSD(R) to the expected RSD(R) for the concentration measured. Ideally, it should not exceed 2.00
Cochran outlier = a lab that has a within-lab variance on duplicate samples that exceeds a critical value. This means that the lab did not replicate the duplicates well.
Grubbs outlier = outliers whose average values for a sample are either too high or too low and exceed a critical value.

Appendix 2

Monoclonal Antibody Method to Determine Dextran in White Sugar

Immunochemical analysis of dextran in refined sugar – Analytical procedure using antibody for rapid dextran test – Method modified by SPRI for white sugar – Midland Test Kit

PURPOSE: Dextran in white sugar causes crystal distortion and quality problems in the production of hard candies. This test provides a rapid measurement of dextran in white sugar. The method has been modified slightly from the Midland instructions for cane juice and raw sugar to better measure the smaller concentrations of dextran in white sugar.

THEORY: This test is based upon the specific binding of a monoclonal antibody to dextran, causing the dextran to come out of solution. The presence of dextran is determined by measuring the amount of turbidity formed using a Nephelometer (Turbidimeter). The turbidity formed is directly proportional to the amount of dextran in the test sample over the specified test range. The concentration is determined by comparing the turbidity formed in the sample with the turbidity formed with a standard dextran (Amersham Biosciences Dextran 2000).

PROCEDURE SUMMARY USING MIDLAND TEST KIT

1. Prepare Buffer Solution from M9008 powder.
2. Prepare Dextran Standard Solution at 100 ppm from M9018 powder.
3. Prepare Antibody Solution from M9010 freeze dried powder.
4. Set the MCA SucroTest™ Meter to Standard Mode.
5. Pipette 2.0 mL of Antibody Solution into the cuvette and take a blank reading.
6. If the blank is > 80 NTU, filter the entire batch of Antibody Solution. Repeat until < 80 NTU.
7. Standardize the Antibody Solution using the 100 ppm Dextran Standard Solution.
8. Test the Standard and calculate the Conversion Factor (CF) for NTU to ppm Dextran.
9. Prepare a 40 Brix solution of white sugar.
10. Filter the sample on a 0.45μm membrane cartridge filter.
11. Mark the top of one face of a clean cuvette with a permanent marker.
12. Pipette 2.0 mL of Antibody Solution into the cuvette and take a blank (N_0) reading.
13. Add 50μL of filtered sample to the 2.0 mL of Antibody Solution in the cuvette.
14. Mix the cuvette contents, place in the MCA SucroTest™ Meter, then start a 1 minute timer.
15. At exactly 1 minute, take the N_1 reading in NTU.
16. Calculate the Δ NTU = N_1 - N_0
17. Convert the Δ NTU to ppm Dextran = Δ NTU X Conversion Factor (CF).

REAGENT PREPARATION
1. Prepare the Buffer Solution as follows:

   Dissolve the contents of one vial of Midland MCA phosphate Buffer Powder in distilled water and make up to 100 mL.

2. Prepare Dextran Standard Solutions as follows:

   Dextran Standard 2000 is included in the kit. Each vial contains 1.00 gram of powdered standard. Carefully measure 100 mg of the dextran and dissolve it in a 1 liter volumetric flask using distilled water to give a final concentration of 100 mg/L. Make a note of the exact weight of dextran used. Use a magnetic stirrer to ensure complete dissolution of the dextran. This solution may be stored refrigerated for subsequent use (unless cloudiness appears, then discard).

3. Prepare the Antibody Solution as follows:

   The Midland Antibody is supplied freeze-dried in a sealed vial. The color and quantity of freeze dried powder in the vial may vary depending on variances in batch preparation.

IMPORTANT: Store the vials in a freezer (preferred) or refrigerator until ready for use.

The freeze-dried material is reconstituted to make 12 mL of Antibody Solution, which is enough to perform 6 tests depending on whether filtering may be required. Each batch of Antibody Solution prepared must be standardized with a 100 ppm Dextran Standard. **See Note 1.**

a) Carefully open the Antibody vial (remove the metal seal and rubber cap). Transfer 6 mL Buffer Solution into the Antibody vial, replace the rubber cap, shake gently and let sit until the powder is dissolved. Pour the liquid contents of the vial into the wide-mouth plastic cup that is provided.

c) Add another 6 mL Buffer Solution to the Antibody Vial. Replace the rubber cap and shake to dissolve any remaining Antibody and pour the contents into the wide-mouth plastic cup and mix. Discard the glass Antibody Vial. Let the solution stand for a total of 30 minutes. **See Note 2.**

d) Pour the prepared Antibody Solution into the amber vial provided. The Antibody Solution may be kept at room temperature for 4 hours without loss of activity and is stable overnight if refrigerated. Store the antibody in the Amber Vial. If you do not expect to use the Antibody Solution within 24 hours, place it in a freezer. Thaw before use, bring to room temperature, and check for turbidity. Turbidity may develop in the stored solution, and this turbidity must be removed by filtering before use. **See Note 3.**

SAMPLE PREPARATION
This test requires liquid samples. Prepare a 40 Brix (% wt/wt) solution of whites sugar. Example: 10 g sugar + 15 g water. Determine the exact Brix of the sample.

ASSAY PROCEDURE

1. Set the Midland MCA Turbidimeter (nephelometer) to "STANDARD MODE". Refer to the instrument Instruction Booklet to ensure it is set in the correct mode and is properly calibrated. **See Note 4.**

2. All samples to be tested must be filtered prior to assay using a 0.45 μm cartridge filter and a clean 5 mL syringe. Only a few drops of filtered sample are needed. Discard the cartridge filters after use.

3. When handling the cuvettes, do not touch the sides. This may leave oil residue from your fingers that will interfere with the light transmission through the cell. Handle the cuvettes by carefully picking them up touching only the edges near the top of the cuvette. **See Note 5.**

4. Use a permanent black marker to place a small mark such as a (•) on one face of the cuvette near the top edge. Using the 1000μL (1.0 mL) fixed volume pipette (**See Note 6**) with a new disposable tip, transfer 2.0 mL of Antibody Test Solution into a clean cuvette. Press the tip against the side of the cuvette, above the liquid, and depress the plunger slowly so you do not create any bubbles in the liquid.

5. Position the turbidimeter on a flat, solid surface. Carefully wipe the cuvette, if necessary, being careful not to scratch it with the wipe. Then, insert into the chamber in the turbidimeter. Make sure that the mark (•) on the cuvette is positioned to the right of the arrow mark (△) on the instrument case cuvette holder. Note: Always insert the cuvette in this same orientation.

Close the lid and press the READ button. The turbidity in NTU will be displayed within 5 seconds. Press the READ button several times until you get a stable NTU reading (the reading usually drops until stable). Record the NTU reading as the BLANK (N_0).

IMPORTANT: If the N_0 reading is higher than 80 NTU, the total batch of Antibody Solution should be filtered. **See Note 3**. After filtering, start the Assay Procedure over again.

6. Timer needed for this step. Remove the cuvette from the turbidimeter. Add 50μL of prepared filtered refined sugar sample to the same cuvette (**See Note 6**). Cap the cuvette and gently invert 6 times to mix, but avoid trapping air bubbles. Start timer (1.0 minute waiting period).

7. Carefully wipe the cuvette again, if necessary. Then insert into the chamber in the turbidimeter in the same orientation as in step 4. Close the turbidimeter lid.

8. At the end of exactly 1 minute, press the READ button. If you allow the time to exceed 1 minute, the sample must be discarded and the procedure repeated. The turbidity in NTU will be displayed within 5 seconds. Record the NTU reading as the SAMPLE (N_1).

9. Calculate the NTU of Sample as follows:

NTU of SAMPLE (N_1) - NTU of BLANK (N_0) = △ NTU of SAMPLE

10. Use the Calculated Conversion Factor (**See Note 1**), to convert the $\triangle$ NTU of sample into ppm dextran as follows:

$\triangle$ NTU of SAMPLE   X   CONVERSION FACTOR   =   ppm Dextran in solution

To convert ppm Dextran in solution to ppm Dextran on Brix, **see Note 7.**

NOTES

1. Running a Standard Dextran Test to Determine the Conversion Factor

a) For each new batch of antibody solution, prepare a Dextran Standard Solution at 100 ppm per the Reagent Preparation instructions.

b) Follow Assay Procedure Steps 2 - 8 using 50µL of the 100 ppm Dextran Standard Solution as the sample.

c) Use the difference, ($\triangle$) NTU value, between your Blank (N_0) NTU reading and the Sample (N_1) NTU reading to determine the Calculated Conversion Factor:

$$\frac{\text{ppm Standard Solution Concentration}}{\triangle \text{ NTU}} = \text{Calculated Conversion Factor (CF)}$$

Example: Standard $= 100$ ppm; Blank (N_0) $= 42.8$ NTU; Sample (N_1) $= 88.3$ NTU

$$\frac{100 \text{ ppm}}{88.3 - 42.8} = \frac{100 \text{ ppm}}{45.4} = 2.20 \rightarrow \text{ Use 2.2 as the CF}$$

2. If you are going to run more than 10 tests in 24 hours, you can prepare several vials from the same lot all at one time and then perform only one standard test on the batch.

3. If filtering is required, pour the Antibody Solution into the wide-mouth plastic cup. Using a 20 mL plastic syringe, draw 10-12 mL of solution into the syringe. Place a Filter Cartridge on the end of the syringe and filter back into the Amber Bottle. DO NOT press the syringe plunger too hard or you will force solution out of the top of the cartridge. If the filter cartridge becomes plugged, carefully remove the syringe and replace the cartridge with a new one. Continue until all Antibody Solution has been filtered.

4. Refer to the Turbidimeter Instruction Booklet for instructions on setting Standard Mode operation. If the turbidimeter requires calibration, refer to the Instrument Instruction Booklet for the correct procedure. Only use Midland Turbidity Standard M9009. Using any other turbidity standard will cause the meter to calibrate incorrectly. Use 2.0 mL only once and discard. Do not pour back into original bottle. Do not freeze, or expose the turbidity standard to extremely cold or hot temperatures. Keep bottle tightly closed and stored at room temperature.

5. Different sources of cuvettes give different readings, due to either the thickness or clarity of the plastic. This test uses disposable cuvettes so that cleaning and protecting against scratches are

avoided. To retain the accuracy of the test, it is highly recommended that you only use cuvettes supplied by Midland Research Laboratories, Inc., part number M9002. Be careful not to tip the turbidimeter when a cuvette is inside the turbidimeter. This could result in liquid spilling inside the instrument.

6. The Fixed Volume Pipette has two stop settings.   Be careful to only press the plunger down to the first stop when filling. When emptying the pipette, press the plunger to the first stop, pause, and then continue to slowly press the plunger all the way down. If you go beyond the first stop in the fill step, you will extract more than 1.0 mL. The second part of the emptying process ensures that all liquid is expelled from the tip. Depress and release the plunger slowly so that you do not introduce air into the liquid. If this happens, you must start over.

7. To calculate Dextran in the sample as ppm / Brix solids, use the following calculations:

      ①   Δ NTU of Sample X CF = ppm Dextran in solution

Example: Δ NTU of Sample = 10.2; CF = 2.2; Brix = 40.2

          Dextran in solution = 10.2 X 2.2 = 22.4 ppm

$$② \quad \frac{\text{ppm Dextran in Solution X 100}}{\text{Sample Brix}} = \text{ppm Dextran in sugar}$$

Example: $\dfrac{22.4 \text{ X } 100}{40.2} = 55.7$ ppm Dextran in sugar

Report of a Collaborative Study on Turbidity in White Sugar

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Introduction

At the 24th Session, Interim Meeting of ICUMSA, in 2004, in Subject 7, Color, Turbidity and Reflectance Measurement, Recommendation 2 stated: It is recommended to continue the studies on turbidity measurement and to enclose the calibration procedure with formazin standards to the planned collaborative study. A procedure entitled "The Determination of the Turbidity of White Sugar Solutions with Reference to Formazin Turbidity Standards" was published in the report of Subject 7. Associate Referee, Dr. M. Kuchejda, felt that it was important to calibrate spectrophotometers with turbidity standards to compensate for differences in the optical design of photometers, since they are not designed to measure turbid solutions. [1]

A collaborative study on determination of the turbidity in white sugar was undertaken by ICUMSA Associate Referee M.A. Godshall. The method tested was an extension of ICUMSA Method GS2/3-10, White Sugar Solution Colour [without pH adjustment]. The turbidity was determined as the difference, in ICUMSA Color Units (IU), between "unfiltered color" and "filtered color". Associate Referee S. Yilmaz, of Schimdt+Haensch re-wrote the spectrophotometer calibration procedure using latex standards, since hydrazinium sulphate, used to prepare formazin standards, is potentially carcinogenic.

Organization of the Test

Fourteen laboratories agreed to take part in the study. Of these fourteen labs, twelve labs returned valid results, which were used in calculating the results of the collaborative test. The participating laboratories, which represented eight countries, are shown in Table 1.

Table 1. Laboratories that participated in the turbidity collaborative study

> British Sugar PLC, Wissington Sugar Factory, Norfolk, England
> C&H Sugar Company, Crockett, California, USA
> Coca-Cola Services N.V., EU Quality Group, Brussels, Belgium
> Centro de Tecnologia Canavieira/Copersucar, São Paulo, Brazil
> Danisco Sugar & Sweeteners Development Center, Nakskov, Denmark
> InnoSweet GmbH, Braunschweig, Germany
> PepsiCo International, Guangzhou, China
> Rogers Sugar, Ltd., Vancouver, B.C., Canada
> Rogers Sugar, Ltd., Taber, Alberta, Canada
> Schmidt+Haensch GmbH, Berlin, Germany
> Südzucker AG, Dept. Central Analytics, Obrigheim, Germany
> Sugar Processing Research Institute, New Orleans, Louisiana, USA

Each participant was mailed 12 sugar samples of approximately 200 g each. Samples were identified by random 3-digit codes. Participants were requested to determine the color and turbidity of each sample once, using an enclosed method entitled, The Determination of the Turbidity of White Sugar Solutions, adapted from ISBT Procedure 1.0, Dec. 2003, Turbidity, [2] which in turn is similar to ICUSMA Method GS2/3-10, except that it incorporates a turbidity determination. The method, as written, was reviewed by Mr. Geoff Parkin, the Referee for ICUMSA General Subject 2, White Sugar. The spectrophotometric calibration procedure provided by Associate Referee Yilmaz was also provided, and participants were encouraged, but not required, to conduct the calibration procedure and to included the turbidity results corrected according to the determined factor. The sugar samples represented blind duplicates of three beet sugars and three cane sugars, which had been chosen by LGC in England (now a joint venture with Quality Management Company) to represent a range of turbidity. The samples were prepared and mailed by LGC to the participants.

Although it had been discussed about whether to include a specific method for dissolution of samples, in the end, it was decided that participants could use their normal dissolution procedure for determining the color of sugar solutions.

Because of several inquiries, we recommended the use of AMCO Clear Latex Turbidity Standards sold by GFS Chemicals.

Results

Table 2 contains details of the tests used by the participants. Although participants were not asked to provide the type of spectrophotometer used, several returned that information. Please note that the lab numbers are randomized to maintain confidentiality of the reporting labs, and do not correspond with the alphabetized list in Table 1. Labs 5 and 8 were not included in the results because Lab 5 did not return results and Lab 8 used 1.2 μm membranes for filtration.

Table 2. Method details.

Lab	Cell, cm	Dissolution, min	US, time	Instrument	Calibration
1	10	MS, 15 min	3 min	-	No
2	16.3 or 4	OS, as needed	3 min	Index ATM420	No
3	10	MS, 15 min	No	-	Yes - formazin
4	5	MS, 12 min	30 sec	Shimadzu UV Mini 1240	Yes
6	10	MS, 10-15 min	10 sec	Spectronic 1001	Yes
7	10	Wrist shaker, 15 min	15-30 sec	-	Yes
9	10	MS, 4 min	5 min	-	No
10	5	MS, 60 min	No	Perkin Elmer Lambda 25	No
11	10	MS, 30 min	15 sec	-	Yes
12	5	Shaker, as needed	No	-	Yes
13	5	Shaker, as needed	No	Hitachi U 2000	Yes
14	10	MS, 30 min	No	-	Yes

US = Ultrasound
MS = Magnetic Stirrer
OS = Orbital Shaker

Six of the twelve participating laboratories conducted the spectrophotometric calibration procedure.

Table 3 includes the raw turbidity data, with the spectrophotometer not calibrated. Table 4 compares turbidity data of the six labs that calibrated the spectrophotometer. Table 5 presents over-all mean turbidity results of the spectrophotometer calibrated vs not calibrated, for the six labs. Table 6 presents the statistics of the collaborative test, spectrophotometer not calibrated.

Table 3. Raw data of turbidity collaborative study - Spectrophotometer not calibrated.

Lab	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6	
	537	216	468	920	273	739	870	665	590	784	141	345
1	2	3	31	32	47	47	174	180	5	5	6	5
2	3	3	31	31	46	47	165	165	4	3	4	5
3	2	4	29	27	41	38	129	120	4	4	5	5
4	4	4	33	33	54	53	175	158	3	4	8	8
6	3	3	27	28	39	38	139	138	4	4	4	5
7	3	4	33	35	55	47	194	187	5	4	7	5
9	2	3	30	27	38	41	141	163	5	3	3	4
10	3	3	26	26	41	38	149	133	5	4	4	5
11	4	0	29	32	43	38	141	140	5	4	5	3
12	4	4	27	28	42	38	152	154	4	4	3	5
13	2	2	28	29	38	38	144	141	4	4	5	4
14	2	1	24	24	37	35	130	135	3	3	3	4

Outliers are shown as shaded cells.

Comments from participants are included in Appendix 1. Since color data were available, the collaborative study statistics were also calculated for the color results. These are shown in Appendix 2. The turbidity method is presented in Appendix 3.

Table 4. Comparison of turbidity data with the spectrophotometer calibrated (grey cells) vs the spectrophotometer not calibrated (clear cells).

Lab	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6	
	537	216	468	920	273	739	870	665	590	784	141	345
6 - cal	3	3	27	28	40	39	140	139	4	4	4	5
6	3	3	27	28	38	39	139	138	4	4	4	5
7 - cal	3	4	36	37	60	51	210	202	5	4	8	6
7	3	4	33	35	55	47	194	187	5	4	7	5
11 - cal	3	0	29	32	43	38	141	140	5	4	5	3
11	4	0	28	32	43	38	141	140	5	4	5	3
12 - cal	4	4	27	28	44	38	149	152	4	4	3	5
12	4	4	27	28	42	39	152	154	4	4	3	5
13 - cal	2	2	29	30	43	39	149	146	4	4	5	4
13	2	2	28	29	38	38	144	141	4	4	5	4
14 - cal	2	1	25	25	38	37	136	142	3	3	3	4
14	2	1	24	28	37	35	139	135	3	3	3	4
M - cal	3.0	2.3	28.8	30.0	44.2	40.3	154.2	153.5	4.2	3.8	4.7	4.5
M	3.0	2.3	28.0	29.3	42.3	39.2	150.0	149.2	4.2	3.8	4.5	4.3

Table 5. Over-all means of turbidity results, spectrophotometer calibrated vs not calibrated. Data shown are the mean of six labs.

Treatment	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Calibrated	2.7	29.4	42.3	153.9	4.0	4.6
Not Cal	2.7	28.7	40.8	149.6	4.0	4.4
Dif (C-NC)	0	0.7	1.5	4.3	0	0.2
% Dif	0	2.4	3.6	2.9	0	4.5

Table 6. Statistics of the collaborative test for turbidty: Spectrophotometer not calibrated. Samples are arranged in order of lowest to highest turbidity.

Sample	1	5	6	2	3	4
No. Labs	12	12	12	12	12	12
Labs retained	10	12	11	12	12	12
Lab(s) rejected	Labs 3, 11	0	Lab 4	0	0	0
Mean	2.9	4.0	4.5	29.1	42.5	152.8
s(r)	0.4	0.6	0.9	1.1	2.3	5.6
s(R)	0.9	0.7	1.0	3.0	5.8	20.0
RSD(r) %	15.42	15.15	20.65	3.64	5.41	3.67
RSD(R) %	29.97	17.16	22.57	10.39	13.62	13.11
r	1.3	1.7	2.6	3.0	6.4	15.7
R	2.4	1.9	2.8	8.5	16.2	56.1
HORRAT	2.20	1.32	1.77	1.08	1.50	1.75
Sugar type	Beet	Cane	Cane	Beet	Beet	Cane

Note: HORRAT is not relevant for turbidity studies, but is included for illustrative purposes only. See discussion.

Discussion

Results from Lab 3 and Lab 11 were identified as Cochran outliers for Sample 1. Cochran outliers are those that have maximum within lab variance - that is, the analyst did not have good repeatability on duplicate samples. The one other outlier (Lab 4, Sample 6) was identified as a high Grubbs outlier, indicating results that were "too high" compared to the mean, causing an increase in the variance above a critical point.

Tables 4 and 5 evaluate the effect of calibrating the spectrophotometer. It is readily noted that calibrated and non-calibrated results are very similar. The over-all sample means of calibrated vs non-calibrated varied by less than 5% (Table 5). From this data it was possible to conclude that calibrating the spectrophotometer is not required for routine determination of turbidity.

The statistics of the study are shown in Table 6. The Horwitz Ratio (HORRAT), which is a way to evaluate the relative standard deviation of the reproducibility [(RSD(R)] of a study compared to the expected results is included for illustrative purposes only since this ratio cannot be used to evaluate non-chemical data because a weight unit is required for its determination. Therefore, we made the assumption that turbidity data may be similar to other white sugar impurities, such as sediment, that are in the ppm range in order to create a presumptive HORRAT ratio. Another way to evaluate the results of the RSD(r) and the RSD(R) in nonchemical data is to choose an amount of variation that is acceptable in laboratories. For evaluation of results of the 2004 color collaborative study, Geoff Parkin chose 5% for RSD(r) and 10% for RSD(R).[3] His overall RSD(r) was 4.89% and the over-all RSD(R) was 8.15%, which met the pre-determined criteria.[3]

The turbidity results in this study showed high variability in repeatability and reproducibility in the low turbidity samples (1, 5, and 6) and less in the higher turbidity samples (2, 3, and 4). The low turbidity samples were well below the maximum 20 IU turbidity allowed by the International Society of Beverage Technologists (ISBT), but the other three results were considerably higher and would cause the white sugars to be rejected. [3]

The over-all mean RSD(r) for turbidity in this study was 10.66% and the over-all mean RSD(R) was 17.80% (Table 7a). (It is recognized that it is not strictly valid from a statistical viewpoint to average these parameters, but it is a useful indicator nevertheless.) These results indicate that turbidity variance is about double that for color variance based on the 2004 collaborative study of Parkin.[2] In some earlier comparative studies in our laboratories in cooperation with Pepsi Cola , we also noted that the turbidity variance tended to be higher than that of color. The average HORRAT, given the assumptions made, was 1.60, within the acceptable guideline of HORRAT not to exceed 2.00.

Table 7b gives an overview of the color variability. The color RSD(r) of 5.18% is within the guidelines for acceptable repeatability established by the 2004 collaborative study. However, the RSD(R) of 19.23% is double the established 10%. These results confirm the observation of higher variability in very low color white sugars. ISBT guidelines for white sugar color are maximum 35 IU for beet and 45 IU for cane, so all of these sugars were in the acceptable color range.[3]

ISBT Guidelines (see attached method) specify repeatability no greater than 3 and reproducibility no greater than 5 for sugars with turbidity up to 20 IU. From Table 6, it can be seen that these criteria are met for all samples (1, 5, 6) with turbidity less than 20.

Table 7a. Overview of turbidity variability

Sample No.	Turbidity, IU	RSD(r)%	RSD(R)%	HORRAT
1	3	15.42	29.97	2.20
5	4	15.15	17.16	1.32
6	5	20.65	22.57	1.77
2	29	3.64	10.39	1.08
3	43	5.41	13.62	1.50
4	153	3.67	13.11	1.75
Mean	--	**10.66**	**17.80**	**1.60**

Table 7a. Overview of color variability

Sample No.	Color, IU	RSD(r)%	RSD(R)%	HORRAT
1	4	6.93	38.03	2.97
6	7	10.91	24.25	2.03
5	8	5.84	19.27	1.65
4	21	2.57	13.62	1.35
2	29	2.53	7.32	0.76
3	33	2.30	12.91	1.37
Mean	--	**5.18**	**19.23**	**1.69**

Conclusions

Based on these results, it is possible to conclude that calibration of the spectrophotometer is not necessary to reproducibly determine turbidity in white sugar solutions. This eliminates a level of complexity in the test, making it easier for field laboratories to conduct the method.

The results confirmed that turbidity results have a variability about double that of color results -- 10% for RSD(r) (within lab repeatability) and 20% for RSD(R) (among labs reproducibility).

Acknowledgments

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APPENDIX - 1

Comments from Participants

Lab 1. This lab has made detailed studies of the dissolution of white sugar and detailed their procedure: Magnetic stirring for 15 min; then 2-3 min in an ultrasonic bath; after pouring the sample into the measuring cell, they hold it in the cell for 15 min before reading. "Sample no. 870 had a very unstable reading."

Lab 2. Used a 4 cm cell for samples 665 and 870 because of high turbidity.

Lab 3. This lab calibrated the spectrophotometer with formazin samples but did not calculate the correction factor. They calculated turbidity and color with both 5 cm and 10 cm cells. The 10 cm cell results were used.

Lab 4. This lab obtained the latex turbidity standards but found that their turbidities were extremely low. It appeared that the standards may have deteriorated, probably from shear stress due to mistreatment in transit.

Lab 5. This lab used 1.2 μ filters instead of 0.45 μ filters. "Some samples difficult to filter." Results were not included in the statistics for the turbidity collaborative study.

Lab 6. This lab conducted the test using both the laboratory spectrophotometer and the process spectrophotometer and calibrated both. The process spectrophotometer gave lower color and turbidity results than the lab spec, but on comparison with the results of the collaborative study, we feel that the variation is within the expected variation for reproducibility. We used the results from the laboratory spectrophotometer. "Sample 273 had black specks."

Lab 7. "Found absorbance very low and hard to obtain stable results."

Lab 8. Did not return results.

Lab 9. No comments.

Lab 10. No comments.

Lab 11. No comments.

Lab 12. No comments.

Lab 13. No comments.

Lab 14. No comments.

APPENDIX 2 - Color Results

Table 1/A2. Color results from the turbidity collaborative study - Method GS2/3-10

Lab	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6	
	537	216	468	920	273	739	870	665	590	784	141	345
1	4	4	29	28	33	31	20	21	7	7	6	6
2	4	8	29	30	30	31	22	24	9	16	6	8
3	3	3	27	28	29	29	18	18	7	7	6	5
4	1	1	26	27	30	31	18	16	6	5	4	4
5*	8	8	32	33	36	36	24	26	8	8	7	6
6	7	6	33	32	37	37	20	24	11	11	10	9
7	7	6	38	39	44	43	36	34	11	16	10	10
9	6	6	30	29	35	34	22	23	9	8	8	7
10	3	3	26	25	27	26	16	16	6	7	7	6
11	4	7	29	29	32	33	22	22	8	9	7	9
12	8	4	30	30	34	34	22	23	9	9	8	7
13	4	8	26	30	30	32	20	26	8	8	6	6
14	4	4	30	29	33	32	20	20	8	8	7	6

* Lab 5 used 1.2 µ filters, but the color results were used because the results are within the means of the over-all test and did not contribute significantly to the variability (that is, statistics are essentially unchanged with or without the results included); and also this was not an official collaborative test for color determination, only for turbidity. Turbidity results from this lab were not used in the statistical evaluation.

Outliers are shown as shaded cells

Table 2/A2. Statistics of the test - Color

Sample	1	2	3	4	5	6
No. Labs	13	13	13	13	13	13
Labs retained	12	12	13	12	13	13
Lab(s) rejected	Lab 11	Lab 7	0	Lab 7	0	0
Mean	4.2	29.1	33.0	21.0	8.2	7.0
s_r	0.3	0.7	0.8	0.5	0.5	0.8
s_R	1.6	2.1	4.3	2.9	1.6	1.7
RSD_r %	6.93	2.53	2.30	2.57	5.84	10.91
RSD_R %	38.03	7.32	12.91	13.62	19.27	24.25
r	0.8	2.1	2.1	1.5	1.3	2.1
R	4.4	6.0	11.9	8.0	4.4	4.7
HORRAT	2.95	0.76	1.37	1.35	1.65	2.03
Sugar type	Beet	Cane	Cane	Beet	Beet	Cane

Note: HORRAT is not relevant for color studies, but is included for illustrative purposes only. See discussion.

APPENDIX 3

THE DETERMINATION OF THE TURBIDITY OF WHITE SUGAR SOLUTIONS

ABSTRACT
The method to determine the turbidity in white sugar is derived from ICUMSA Method GS2/3-10 (2005), The Determination of White Sugar Solution Color [without pH adjustment] - Official. In summary, the turbidity is measured as the difference in the color of a white sugar solution before filtration and after filtration. The turbidity thus measured is reported as ICUMSA color units (IU).

Field of Application: This method is limited to sugars with color up to 50 IU. This method is not to be used when pH adjustment is required, e.g. when color exceeds 50 IU

PRINCIPLE

White sugar is dissolved in distilled water to give a 50 Brix (% wt/wt) sugar solution. The absorbance and color of the solution are determined. The solution is then filtered through a membrane filter to remove turbidity. The absorbance and color of the filtered solution are again determined. The difference of the two color measurements constitutes the turbidity. Absorbance is measured at 420 nm.

REPRODUCIBILITY

Based on preliminary studies, for sugars with turbidity values up to 20 IU, the repeatability of two results should not be greater than 3 IU. (Repeatability refers to how well an analyst can repeat his results.) The reproducibility of two results should not be greater than 5 IU. (For reproducibility conditions, the results of two different laboratories for the same sample should not deviate more than 5 IU from each other.)

EQUIPMENT

A.   Spectrophotometer. Spectral bandwidth no greater than 5.0 nm, wavelength increment 1.0 nm, wavelength accuracy $\pm$ 0.3 nm; capable of accommodating a cell at least 5 cm in length and preferably 10 cm.

B.   Optical cells. Use a cell of at least 4 cm length. Cells of 10 cm length are preferred for very low color white sugars. Different cells should be within 0.2% of being identical to each other in readings. Other cell lengths, such as 5 cm, may be used, with the appropriate change in the calculation noted.

C.   Membrane filters. Pore size 0.45 μm; diameter 47 mm; cellulose nitrate.

D.   Laboratory balance. Readable to 0.1 g.

E.   Brix Refractometer

F.   Membrane filter holder

G.   Vacuum source for filtration.

H.   Ultrasonic bath. (Optional) For de-aerating sugar solutions.
I.   Magnetic stirrer.
J.   Assorted laboratory glassware. Beakers, filter flasks & stirring bars.

REAGENTS
Distilled water or water of equivalent purity.

PROCEDURE

1. Prepare a 50 Brix white sugar solution (50 g sugar + 50 g water). Stir with a magnetic stirring bar, at room temperature, until the sugar is thoroughly dissolved. (Concentration will be measured by refractometer, which compensates for slight deviations in weighing.)

2. If the solution is full of bubbles, slow stirring will usually remove them, or letting the beaker sit for a while. If this does not work, de-aerate in an ultrasonic bath for 3 minutes. Bubbles are not usually a problem when preparing white sugar solutions.

3. Measure the Brix of the solution on a Brix refractometer. It is vitally important that this step be carried out, as even very small differences in weighing can change the concentration, and, hence, change the results. For further details refer to ICUMSA Method GS4-13.

4. Zero the spectrophotometer at 420 nm with deionized or distilled water.

5. When using re-usable optical cells, rinse the cell with a small amount of the solution to be measured, making sure that the outside of the cell remains clean and dry.

6. Measure the absorbance of the unfiltered sugar solution at 420 nm in a cell of appropriate length for the color of the solution.

7. Calculate the color of the unfiltered solution, using the calculation provided below.

8. Filter the remaining solution through a 0.45μm membrane, with vacuum.

9. Measure the Brix of the filtered solution.

10. Read the absorbance of the filtered solution at 420 nm using the appropriate cell. Calculate the color of the filtered solution, using the calculation provided below.

11. Express the results to the nearest whole number.

Calculations for Color and Turbidity.

Obtain the concentration, based on the refractometer reading, of total solids (g/cm^3) from Table SPS4 of ICUMSA Methods (1994) or its update.

$$\text{Unfiltered Color} = \frac{\text{Abs}_{420nm \text{ (unfiltered solution)}} * 1000}{(\text{Cell length, cm}) * (\text{Conc total solids, g/cm}^3)}$$

$$\text{Filtered Color} = \frac{\text{Abs}_{420nm \text{ (filtered solution)}} * 1000}{(\text{Cell length, cm}) * (\text{Conc total solids, g/cm}^3)}$$

Turbidity = (Unfiltered Color) - (Filtered Color)

Note: Filtered color is the color of the sugar and is comparable to the value obtained with ICUMSA Method GS2/3-10 (2005).

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ISBT Guidelines for Granular Sucrose, 2004, ISBT Procedure 1.0, Turbidity, Dec. 2003
ICUMSA Method GS2/3-10 (2005)
ICUMSA Method GS4-13.
ICUMSA Table SPS4

Last update: May 17, 2006

Elimination of Microorganisms Capable of Growing at High Temperature at Early Stages to Achieve Significant Advantages in Sugar and Alcohol Industries

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Abstract

Growth of microorganisms capable of growing at high temperatures is often ignored in sugarcane milling factories. Although there is significant amount of work done on this subject in diffuser factories; the significance of killing them and its impact till final molasses is seldom mentioned. Killing rather than inhibiting growth of these and mesophilic microbes should give significant impact during further processing. Chemicals used for controlling microbes must have the ability to kill them in dormant phase, should show results in molasses and help in better performance of yeast during fermentation. These chemicals must have the ability to perform in cane juice in a very short time and on dormant microbes as well as actively growing microbes. This is possible only for chemicals that act on various enzyme systems including respiration. Carbamate or dithiocarbamate based biocides do have potential to kill actively growing and dormant microbes in cane and beet juice conditions. They are "safe" to use in the sugar industry for mill sanitation as they are destroyed to non – toxic products by heat in few minutes. The only difficulty is that they require more time to act, but this can be eliminated by using formulations of synergistically acting compounds and some natural and or inert compounds to make them perform quickly to kill most microbes in less than available time i.e. within 10 minutes for mills and 1 minute for factories having diffuser. This time frame is most important as even dormant microbes that have the ability to grow at high temperature must be killed before the juice is heated or prior to its entry into the juice heater for mills and prior to diffuser entry. Carbamates will be destroyed after this stage, and thermophilic bacteria will initiate growth after this stage. Using such formulations not only reduces inversion and acid formation at mills but also reduces destruction of reducing sugars during clarification and further process (in diffuser as well) resulting in reduction in the microbial population in sugar and molasses. This lower microbial count of sugar and molasses improves the keeping quality of both; especially molasses and helps in obtaining better fermentation by reduction of competition for sugars by microbes with yeast. Data of 10 sugar factories with mills and one factory with diffuser is discussed.

Introduction

Sugarcane juice contains many components that inhibit action of many disinfectants or biocides. Amino acids, proteins and other organic matter inhibits action of oxidizing disinfectants like chlorine, ozone and aldehydes and also inhibits non-oxidizing biocides like quaternary ammonium compounds which are also inhibited by calcium present abundantly in cane juice.

The other crucial factor is that the time available for the biocide to kill microbes is very short, especially when one intends to use a biocide for diffusers. Mesophilic microbes growing at normal temperature grow rapidly form the time of harvest and their growth is boosted after the shredder and continues in primary / mixed juices until the juice is heated above 60^0 C.

Dithiocarbamates do have the capacity to work as biocides under cane juice conditions and they are converted to non-toxic products immediately when temperature exceeds 60^0 C, and there are no traces left even in clear juice. Thus dithiocarbamate based or its formulations are safe to be used as biocides for sugar mill sanitation. The only drawback is the lack of penetration, and thus they require more time to kill most of the microbes found in cane juice. Due to presence of inhibiting components, even the action of quaternaries is reduced. Figures 1 and 2 are the plots of killing efficiency of quaternary (BKC) and dithiocarbamate biocides respectively. From these, it is clear that only specially developed formulations have ability to kill about 90% microbes when used at 10 ppm conc. in cane juice within the time limit we have in sugar mills – about 15 minutes and in diffusers having just couple of minutes for juice to achieve high temperature.

Heating or even boiling of juice to 100^0 C does not kill all microbes as assumed, but it changes the microbiology. Although many normal bacteria are killed, appreciable numbers do survive, but remains in a dormant stage waiting the return of favorable conditions! Microbes having the capacity to grow at high temperatures which are present in small number in the dormant stage in cane juice find temperatures suitable for their growth and start multiplying and consume sugar. The only chance we have to minimize them is to kill most of them in cane juice where they are in the dormant stage. As metabolism during dormancy is minimal, it is very difficult for "normal" biocides to kill them. However, certain natural penetrating agents do have the capacity to take biocide into dormant cells and cause cell death.

Materials and Methods

A. Laboratory Evaluation

Killing efficiency of various biocides, quaternary BKC 50% active, Na-N-dimethyl dithiocarbamate (40%), combination with ethyl dithiocarbamate 40% active as given in The Cane Sugar Handbook by Mead and Chen, Polmax ESR, 40% active formulation of dithiocarbamates and Polmax Supreme also 40% formulation of dithiocarbamates both formulations have rapid action (patented in India) were taken in the prescribed doses in 500 ml cane juice in 1 liter conical flask; microbes were estimated using the plate count method using nutrient Agar supplemented with 10% cane juice. The microbial count was also taken indirectly using impedance / capacitance measurement.

B. Factory Evaluation

The special formulations were used for mill sanitation for a period of 15 days in every factory after the estimation of sugar losses during the use of other biocides that factory was using as per the prescribed dose by the respective supplier. The evaluation involved estimation of reducing sugar and acidity by titration of primary juice, mixed juice, clear juice, syrup and final molasses. The data were computed as per the method given in ICUMSA Proceedings (Atlanta 2004).

Results and Conclusions

Table 1 gives the comparisons of the rise in reducing sugars and acidity from primary juice (PJ) to mixed juice (MJ) and Table 2 gives comparisons of the rise in reducing sugars and acidity to molasses. The data is of 16 randomly selected Indian sugars factories. Thus this gives comparison of Polmax ESR with almost all leading biocides used in India. From these tables it becomes evident that:

- Irrespective of geo-climatic conditions and use of other types of biocides, special formulation biocide Polmax ESR shows significant reduction in rise in RS and acidity during milling.
- This clearly proves killing of bacteria essential for such conversion of sucrose due to "timely" killing action of formulation.
- There is significant reduction in RS and Acidity in molasses and concomitant reduction in rise in RS and acidity after clear juice.
- This significant reduction in RS and especially acidity indicated killing of microbes capable of producing acid at high temperature due to use of special formulation.
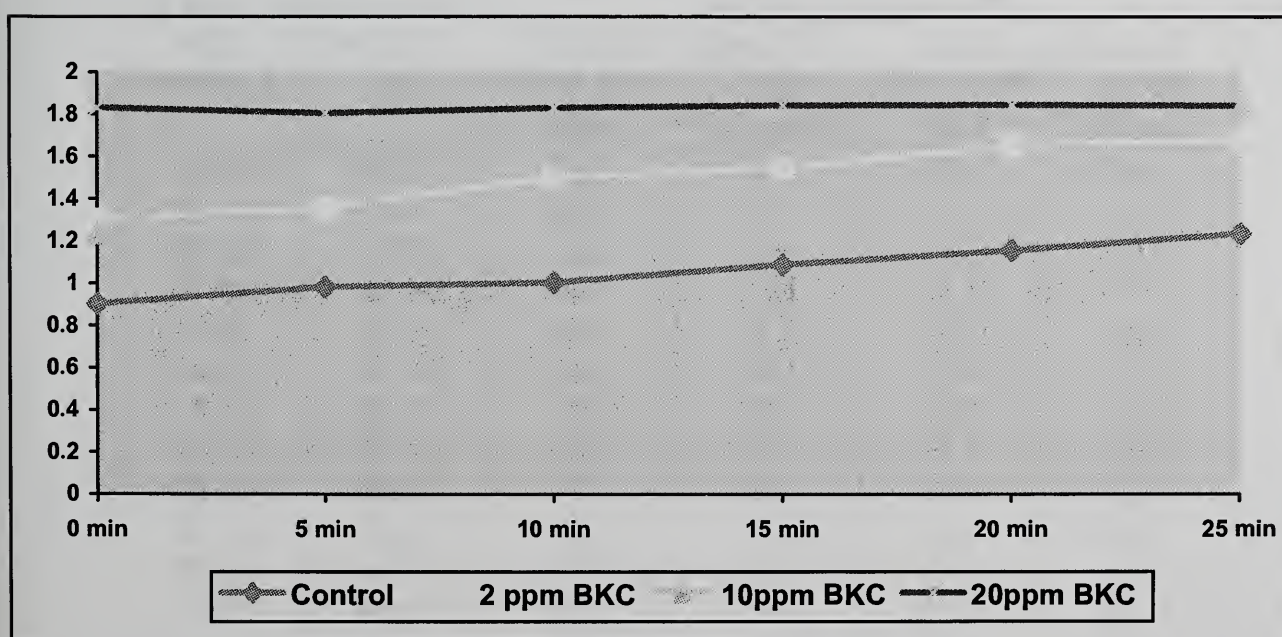

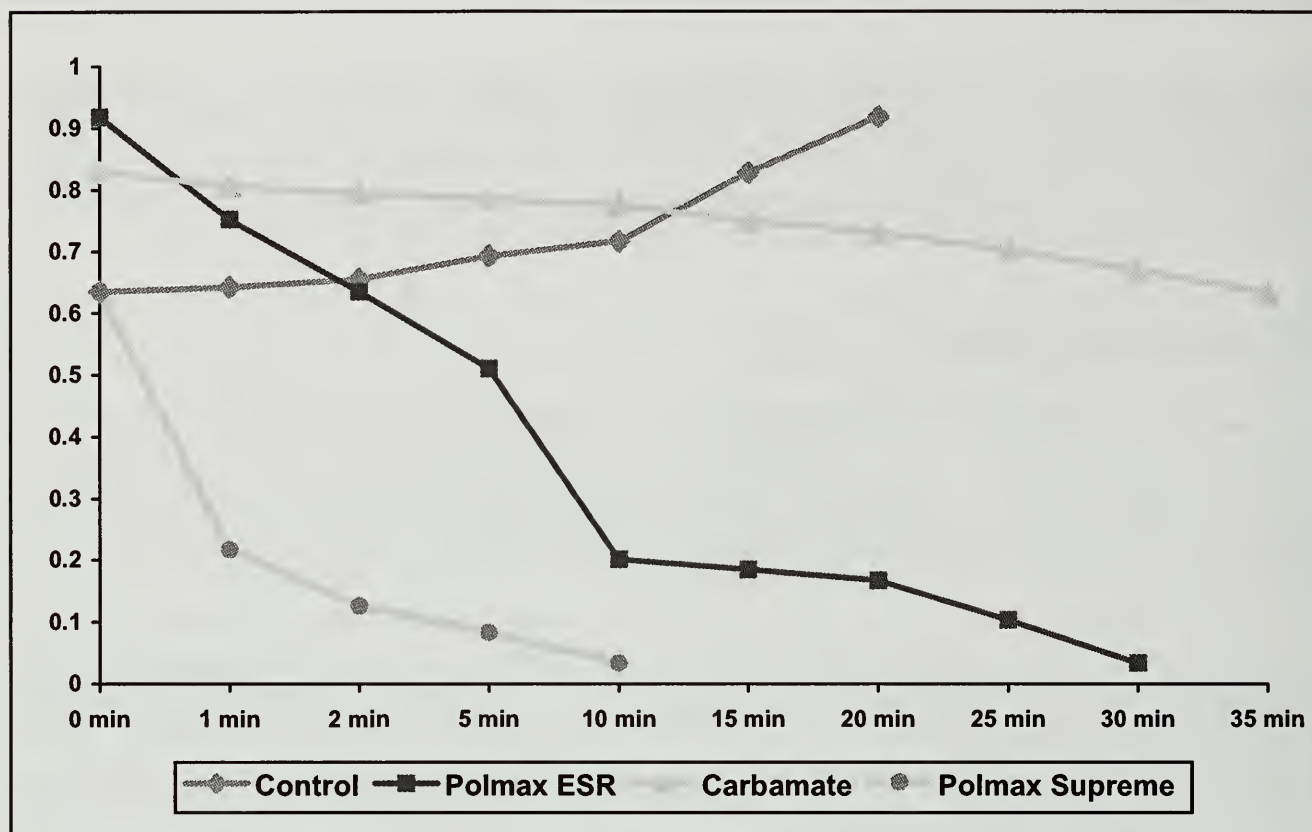**Figure 1.** Killing efficiency of BKC

Figure 2. Killing efficiency of dithiocarbamates.

Table 1. The results at mills.

Factory No.	Rise from PJ to MJ				% Reduction due to POLMAX – ESR	
	Reducing Sugar		TITRABLE ACIDITY			
	Other biocide	POLMAX ESR	Other biocide	POLMAX ESR	RS	ACIDITY
1	2.28	0.48	5.14	2.86	78.90	44.40
2	1.15	0.52	2.79	1.38	54.80	50.50
3	0.81	0.26	1.55	0.21	67.90	86.50
4	0.68	0.31	1.89	0.66	54.40	65.10
5	0.84	0.34	2.12	1.26	59.50	40.60
6	3.08	0.57	1.65	1.02	81.50	38.20
7	0.88	0.38	2.33	0.77	56.80	67.00
8	0.68	0.35	2.38	0.45	48.50	83.80
9	1.20	0.42	2.37	0.26	65.00	88.50
10	1.42	0.40	2.30	0.19	71.80	91.70
11	2.16	0.49	2.38	1.87	77.30	21.40
12	0.58	0.29	1.98	1.00	50.00	49.50
13	1.19	0.36	2.15	0.90	69.70	58.10
14	1.13	0.45	3.21	1.21	60.20	62.30
15	0.65	0.45	2.33	0.85	30.80	63.50
16	1.02	0.35	2.00	0.92	65.70	54.0

Table 2. Effect in Final Molasses.

Factory No.	Rise in RS from PJ to Molasses		Rise in Acidity from CI.J to Molasses		% Reduction due to POLMAX – ESR	
	OTHER	POLMAX	OTHER	POLMAX	RS	ACIDITY
1	11.40	10.46	29.13	21.65	8.25	25.68
2	14.46	9.63	50.90	39.48	33.40	22.44
3	15.49	11.78	24.58	18.26	23.95	25.71
4	12.92	7.63	19.93	16.33	40.94	18.06
5	19.92	17.12	22.27	16.38	14.06	26.45
6	16.27	10.77	14.73	10.86	33.80	26.27
7	10.97	9.80	20.98	15.75	10.67	24.93
8	10.01	8.77	16.98	11.33	12.39	33.27
9	14.01	10.06	14.93	5.08	28.19	65.97
10	23.64	19.05	21.34	17.00	19.42	20.34
11	17.30	13.80	22.37	18.88	20.23	15.60
12	19.91	13.12	24.42	15.38	34.10	37.02
13	20.74	16.29	22.87	19.92	21.46	12.90
14	18.51	12.70	13.15	11.40	31.39	13.31
15	15.78	12.26	17.55	16.10	22.31	8.26
16	16.06	11.81	28.17	22.70	26.46	19.42

Optimisation of Natural Biocides in Beet and Cane Production

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Abstract

Following on from their success in the sugar beet industry, natural biocides such as BetaStab®10 A have been optimised for use in cane mills so they are a cost effective alternative to standard chemical biocides. Leuconostoc species are the main bacterial contamination in cane mills resulting in dextran formation and sugar loss. These natural biocides are fast acting and immediately stop the growth of problem bacteria, so minimising sugar losses and processing difficulties in the mill. Furthermore, these naturally derived products can be used as fermentation aids in ethanol production.

Introduction

A range of natural products (Figure 1) has been found to possess antibacterial activity (Hein, 2006; Pollach and Hein, 2002, 1996) and the search is continuing for further natural products, with the aim of developing a large natural product portfolio suitable for use in a range of applications.
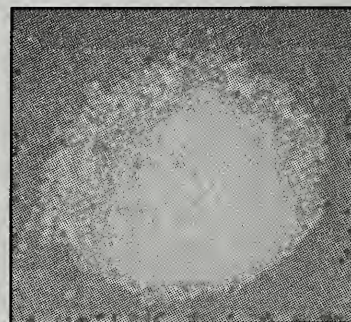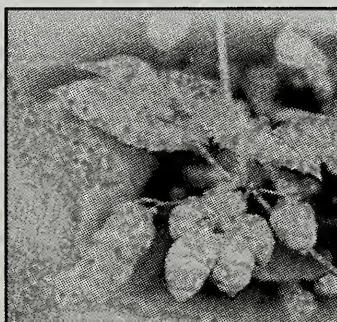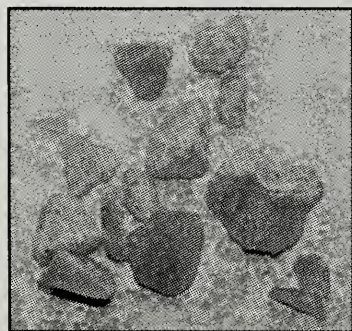

Figure 1. Rosin, hops and palm oil.

The first application for these natural products was developed in the sugar beet industry, as they are particularly active against the main bacterial contaminants found during beet processing. Easy to use water based formulations have been developed, such as BetaStab®10 A, PiStab, PalmStab and PineStab. Further uses for these and other naturally derived products have been established in the sugar cane industry and in ethanol production.

Materials and Methods

Bacterial strains: *Leuconostoc mesenteroides* subsp. dextranicum strain (DSM20187), *Leuconostoc mesenteroides* subsp. mesenteroides strain (DSM20343) and *Bacillus stearothermophilus* (DSM22) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) GmbH culture collection, Germany.

Culture media: Cultures of *L. mesenteroides* were incubated at 30° C under aeration in Man Rogosa Sharpe medium (MRS) (Merck). Cultures of *B. stearothermophilus* were incubated at 60° C under aeration in Brain Heart Infusion medium (Oxoid).

Determination of minimum inhibitory concentration (MIC). The MIC was determined using the broth micro dilution assay. An overnight culture was diluted appropriately to obtain OD 600 = 1.0 (8 x 108 organisms), 30 µl of this was added to 3 ml fresh broth (giving a final concentration of approximately 8 x 106 organisms) and incubated appropriately with controls containing no active organisms. Following incubation, growth quantification was achieved by measuring optical density at 600 nm. The MIC was calculated as a minimum concentration required for a 90% drop in OD from the control.

Results and Discussion

Sugar Beet Industry

The first application for natural biocides was developed in the sugar beet industry. Laboratory tests have shown that these natural compounds are active against the key bacterial contamination such as Bacillus, Clostridia and Thermus species. Against *Bacillus stereothermophilus*, the minimum inhibitory concentration (MIC) for natural compounds compares favourably to commonly used synthetics biocides (Figure 2). Data against other bacterial species have been established. Full-scale commercial trials have confirmed the effectiveness of these naturals as excellent replacements for synthetic biocides.

As well as being safe to handle and dose in the factory, additional benefits when using naturals include lower secondary cost and smoother extraction conditions. Furthermore it has been shown that any residues in molasses do not have any negative effects on yeast growth or ethanol production. In fact, they have potential benefits (see ethanol section).
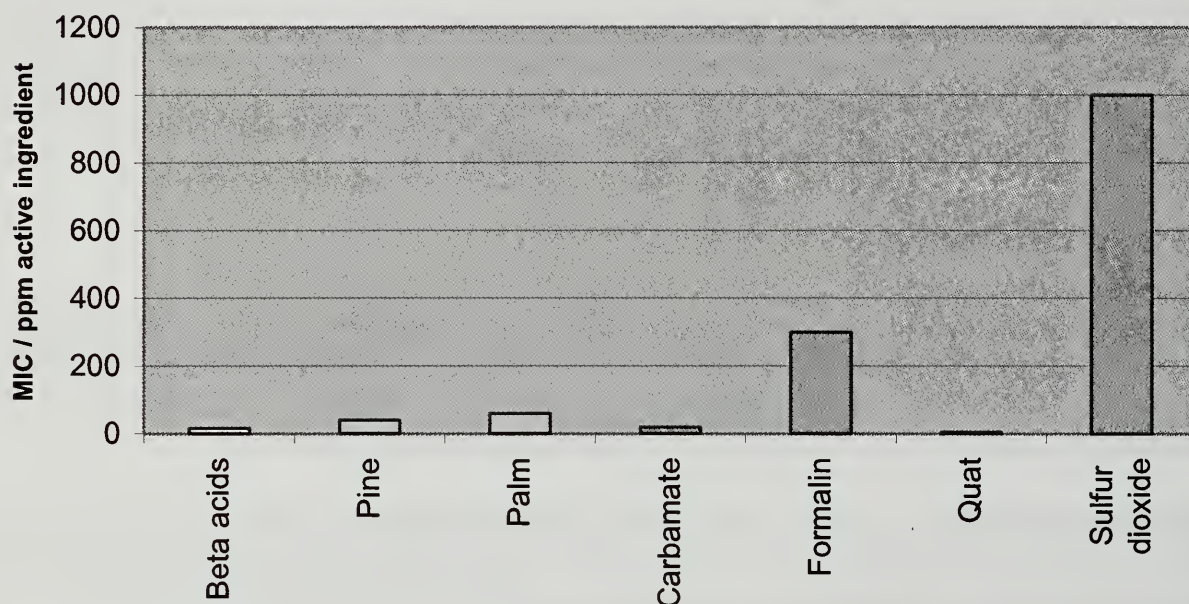
Figure 2. MIC of various natural and synthetic biocides against *Bacillus stereothermophilus*.

Sugar Cane Industry

A range of microbiological contamination is present in sugar cane mills (Trost and Steele, 2002) although by far the main problem is Leuconostoc bacteria. These bacteria are a particular issue, as they produce dextran, which causes sugar losses, increased juice viscosities, slow filtration and poor crystal growth (Ravno and Purchase, 2006). Typical biocides employed against Leuconostoc bacteria include carbamates and quaternary ammonium compounds (Madsen and Day, 2005; Richards, 1999). Hop beta acids show comparable activities (Figure 3) to these synthetic biocides.
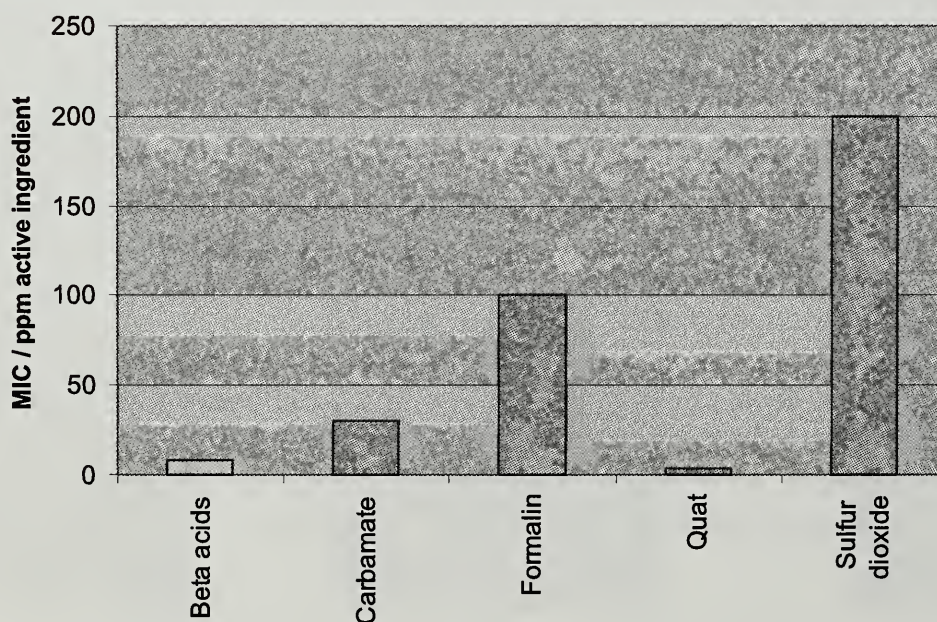

Figure 3 – Activity of hop beta acids and synthetic biocides against *Leuconostoc mesenteroides*.

Dextran is typically used as an indicator of Leuconostoc contamination, although current methods to determine dextran are either long and complicated or too expensive. A good alternative indicator of Leuconostoc bacteria is mannitol and a rapid, easy-to-use and inexpensive method has been developed to measure mannitol in cane juices (Eggleston, *et al.*, 2005). This method has been successfully employed in biocide trials to determine bacterial contamination through the mill train.

A problem with some biocides is that they take many minutes before they start to control the growth of bacteria. Laboratory tests have shown that the addition of 16 ppm beta acids immediately stops the growth of Leuconostoc bacteria (Figure 4). Hence in a factory situation beta acids will be effective as soon as they are dosed.
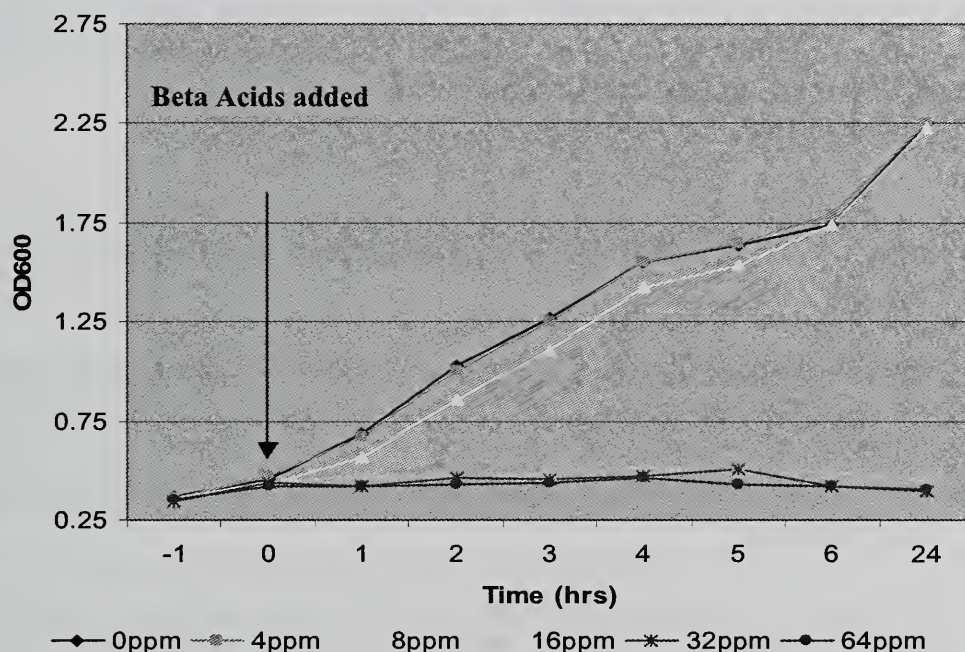

Figure 4. Effect of beta acids on the growth of Leuconostoc mesenteroides

Ethanol Production

It is known that yeast has a high tolerance to hops as they have been used together in brewing for many centuries. Recent work has show that naturally derived hop compounds, at ppm levels, are very good as fermentation aids in ethanol production by maintaining healthy yeast (Rückle and Senn, 2006), which in turn helps to maximise ethanol yields. A range of hop derived products such as IsoStabTM and LactoStabTM have been developed for use in distilleries. The most effective product depends on the feedstock used, and the pH values of the yeast propagation and fermentation tanks. All products are effective in batch, cascade and continuous fermenters and dosing strategies have been optimised for each plant design. Using hops in fermentation can remove the need to use antibiotics such as Penicillin and Monensin. The use of antibiotics is currently being banned in many areas, as it can lead to the build up of antibiotic resistant bacteria, a potential hazard to humans. Antibiotics residues in vinasse or DDG also have the potential to increase antibiotic resistant bacteria through the food chain.

Conclusion

A family of cost effective naturally derived products have been developed. These products can be used in sugar beet and sugar cane processing. As they can have different relative activities against bacterial types, one biocide may not be the most cost effective in all applications. Often a combination of biocides may be necessary to minimise bacterial resistance. Furthermore naturally derived products have been developed as fermentation aids for potable and fuel ethanol production replacing the need to use antibiotics.

Acknowledgments

The authors would particularly like to thank Dr Günter Pollach and Dr Walter Hein of Zuckerforschung Tulln for discovering a range of natural products to be used as biocides in sugar beet processing, and for our continuing collaboration over the past ten years.

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Reducing Analysis Costs:
Development and Adoption of a Silylation Only Sugar Analysis for Cane Payment Purposes

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Durban, 4041, South Africa

A sucrose based cane payment system was introduced into the South African sugar industry in the 1982-83 season. Hourly composite samples of mixed juice are sub-sampled and quickly frozen at -40°C. The samples are composited and analysed on a weekly basis using a gas chromatographic (GC) method to determine the glucose, fructose and sucrose content.

Silylation of the monosaccharides gives rise to a multiplicity of derivatives on the GC, which is undesirable (Figure 1). This is obviated by oximation prior to silylation. Each monosaccharide will then produce only two derivatives Figure 2). The silylation method uses two internal standards (xylose for the reducing sugars and trehalose for sucrose). The method has proved to be accurate and precise, although tedious and costly.

Monosaccharide concentrations are not used for cane payment purposes but are used for factory control calculations. The need for precise, accurate concentrations is not as important for these calculations. A single derivatisation scheme (silylation only) was studied that still produced precise, accurate sucrose figures for cane payment and monosaccharide values for factory control, but was quicker and ultimately less costly. The monosaccharides still give multiple TMS anomer peaks but these co-elute on a megabore column. The first fructose peak and last glucose peak are used for quantitation (Figure 3).
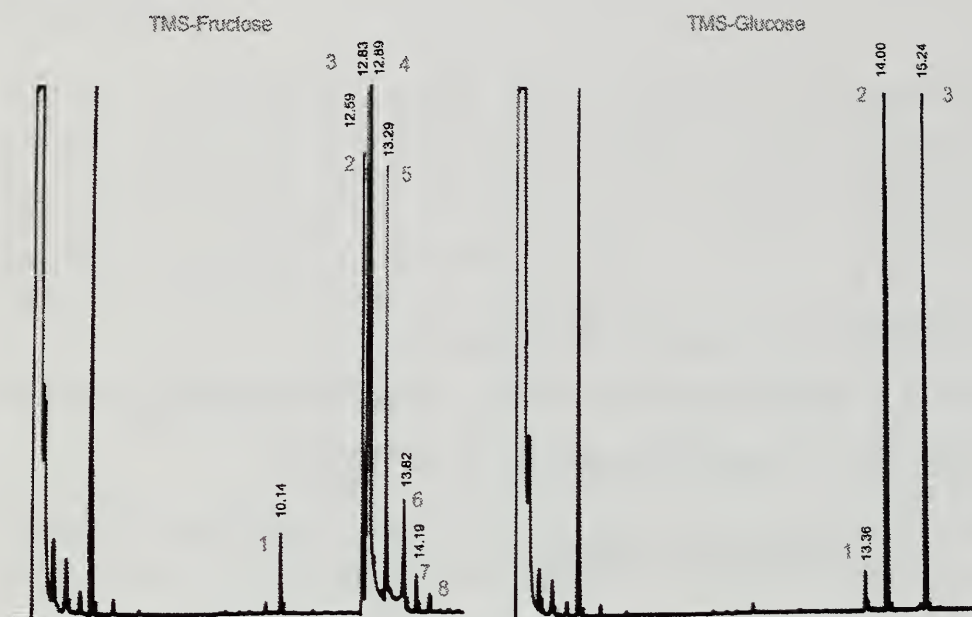
Figure 1. Chromatograms of silylated-only fructose and glucose solutions showing the multiple peaks from the derivatisation. Anomers 5 and 6 of TMS-fructose co-elute with TMS-glucose anomers 1 and 2. Separation on a non-polar 95% dimethyl-5%diphenyl polysiloxane phase capillary column, nitrogen carrier.

Figure 2. Chromatogram of oximated-silylated sugar solution showing the reduced number of monosaccharide peaks (2 each) from the derivatisation. Separation on a non-polar 95% dimethyl-5%diphenyl polysiloxane phase capillary column, nitrogen carrier. Peaks 1=OX-TMS-Xylose; 2a,b=OX-TMS-Fructose; 3a,b=OX-TMS-Glucose; 4=TMS-Sucrose; 5=TMS-Trehalose.

Figure 3. Chromatograms of oximated and silylated xylose, fructose and glucose (upper chromatogram). Silylated only xylose, fructose and glucose (lower chromatogram). Note how on this column the multiple TMS anomers co-elute: the first fructose w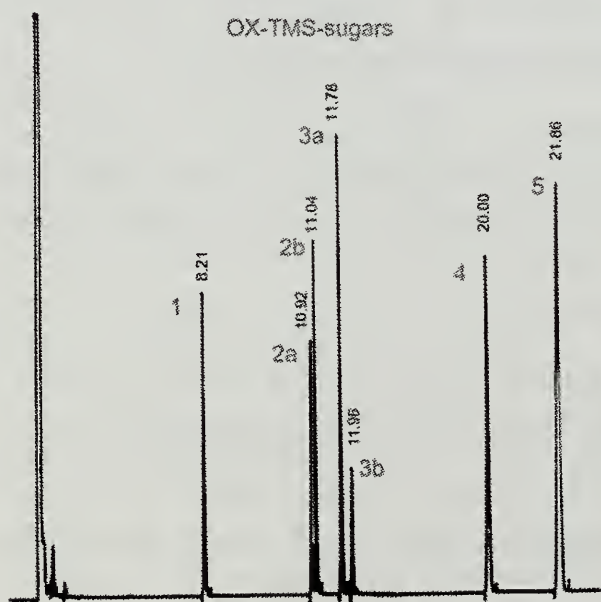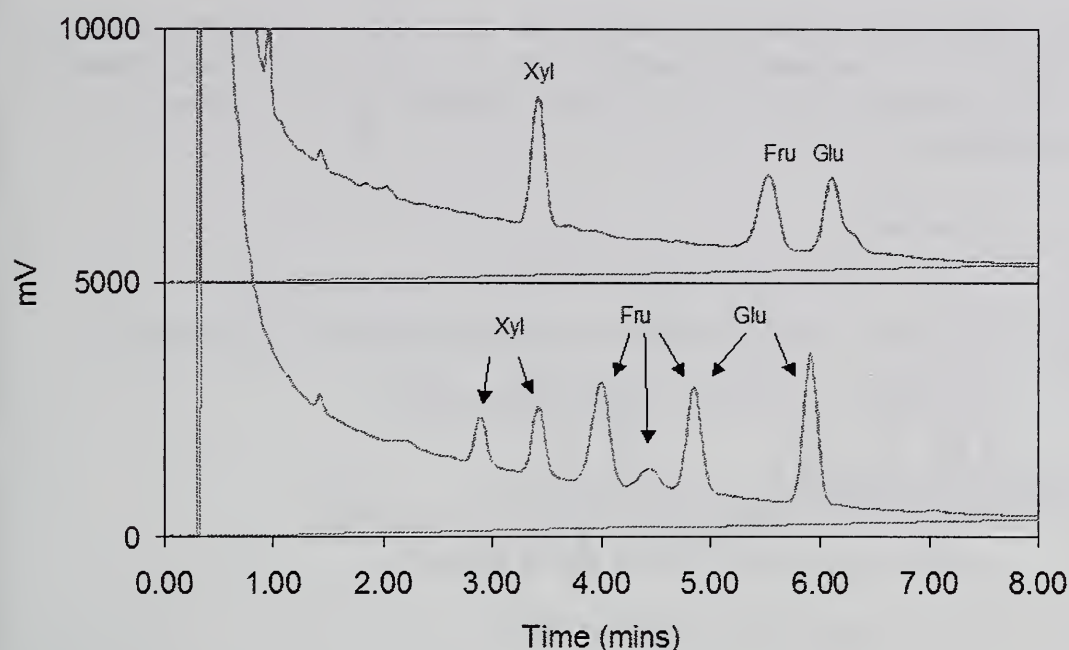ith last glucose. Separation on a non-polar 95% dimethyl-5%diphenyl polysiloxane phase megabore column (0.53mm id), nitrogen carrier.

A comparison of oximation-silylation vs silylation only procedures using trehalose as the only internal standard for sugar was carried out over 2 seasons. Samples were randomly chosen three times during the season (beginning, middle and end) to account for any differences during the season (Table 1).

Table 1. Comparison of oximation/silylation vs silylation only procedures using trehalose as the only internal standard for sugar % MJ (N=160 samples)

Sugar		Oximation/ Silylation	Silylation Only	Difference
Sucrose	Average	10.50	10.49	-0.01
	Range	7.54-12.51	7.53-12.70	
	Std Deviation	1.10	1.10	
	Std error	0.15	0.15	
Glucose	Average	0.26	0.26	0.00
	Range	0.15-0.46	0.15-0.44	
	Std Deviation	0.08	0.08	
	Std error	0.01	0.01	
Fructose	Average	0.27	0.28	-0.01
	Range	0.16-0.44	0.16-0.43	
	Std Deviation	0.08	0.07	
	Std error	0.01	0.01	

A t-test to evaluate the means between the two methods for each sugar showed no statistical difference for the procedures. The concentration range encompasses the normal range found in MJ samples. A plot of the absolute differences for sucrose between the two methods for the second season is shown in Figure 4.
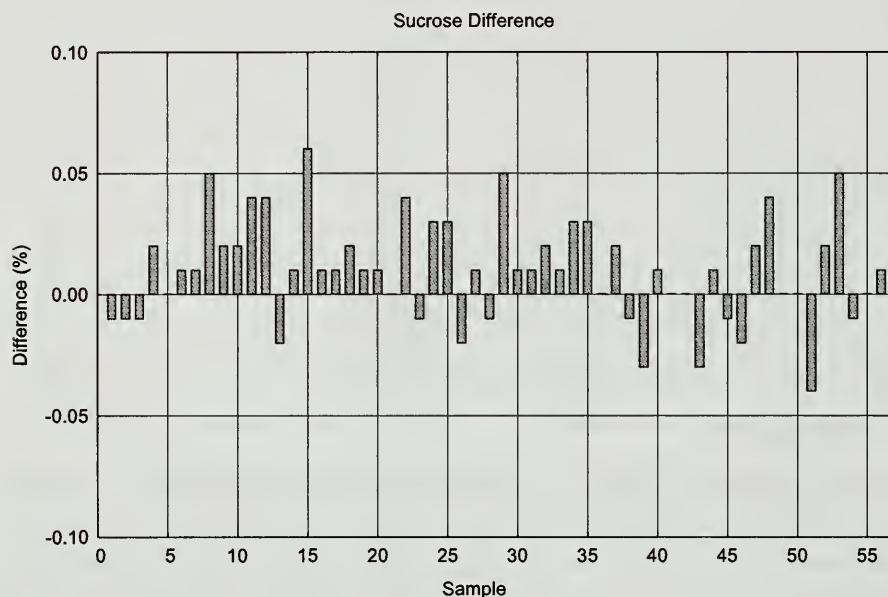
Figure 4. Absolute sucrose difference between the two procedures for during the 2004/05 season.

Advantages of the method include:
- Derivatised samples can be analysed on the same day instead of waiting overnight
- No sample transfer from preparative to analysis vial is necessary
- Savings in consumable costs (no oximation chemicals, glassware & vials)
- Savings in operator time (no oximation preparation, less cleanup)
- GC results available sooner
- More precise results from a single derivatisation (for sucrose)

Conclusions:
- Method was introduced in the 2005/6 season
- Saving of approximately 18% of the cost of the analysis (mainly analyst time)
- The results are available one day earlier
- The customer is happy with the modified procedure

Factory Performance Evaluation with the Advanced Monitoring System (AMS)

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Abstract

During the last decades, automation systems, process control systems, process and laboratory information systems as well as management information systems have been introduced to the sugar industry and have been developed to a high standard today. These tools offer the advantage of much improved data availability/accessibility and a tremendous decrease in labor costs as well as improving factory operations. However, in other industries (e.g., the petrochemical industry) even more advanced expert systems are already state of the art. The "missing link" between the current data and information architecture and the implementation of expert systems is a consistent on-line mass and energy balance of sugar factories.

Introduction

The process control system (PCS) of a sugar factory handles pressures, temperatures, flows and other values that are constantly being measured and controlled at various process areas. Data such as dry substance content (Brix), sugar content (Pol) and purities are determined in the factory's laboratory. Employing data management systems, data from the laboratory and process control system are centrally stored and made available for further evaluations (Vogl, *et al.*, 2000). Until today, the operators had no means to automatically elaborate a complete and self contained mass and energy balance of the factory. The Advanced Monitoring System developed by IPRO Industrieprojekt GmbH and Sugars International LLC (Morgenroth and Weiss, 2003) closes this gap. The Advanced Monitoring System (AMS) automatically imports data from the laboratory and the process control system. Thus, with only roughly 100 process parameters, the whole production process can be balanced regularly and with high precision. The AMS visualizes mass and energy flows of the main stations such as extraction, juice purification, evaporation and crystallization. The operator reaches a much improved transparency of the whole process with the possibility of direct and fast optimization.

Functional Description of the AMS

Figure 1 gives an overview of data processing by the AMS. Data from the laboratory and the process control system are automatically collected in a Process Data Acquisition System (PDAS). The decisive data for the simulation is converted into a XML file (eXtensible Markup Language) and subsequently transferred to the AMS simulation model especially created for each factory. The simulation software is Sugars™ in combination with Microsoft® Visio®. Future applications are intended to export data from the AMS into an Expert system which is able to manage the data for further calculations.
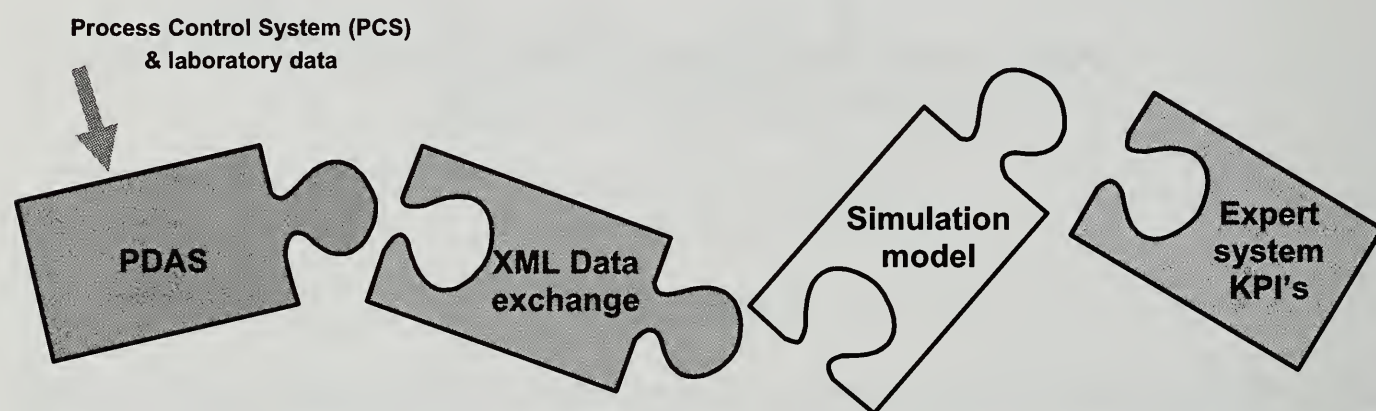

Figure 1. Data import with the Advanced Monitoring System.

Prior to being imported to the Sugars™ model, measured data will be checked for plausibility. If the actual value ranges within laid down tolerance limits, the value will be read in. If it is outside the limits, either the lower or the upper tolerance value will be read, in order to balance at all. Additionally, an "Error report" will be initiated showing breaches of tolerances. Should errors occur during the data exchange between the XML-file and the AMS, this will be displayed as well. Additionally, in Sugars™ the newly read data will be compared with previously processed data establishing a "Comparison Report." and proportional deviations will be calculated and shown. With this, the operator has the ability to observe operational changes.

Figure 2 shows an excerpt from a simulation model of an evaporation plant with 5 effects. The Sugars™ model displays the actual balance of the particular factory. This model shows, among others, the demand of exhaust steam with 128.8 t/h and a pressure of 220 kPa. Apart from that, the mass flows of vapor I to V to each consumer will be verified with actually measured data. Process data, i.e. dry substance contents, temperatures, etc. of juices in the evaporation station as well as condensate and vapor temperatures will be balanced and shown.

Figure 2. Excerpt from a simulation model of an evaporation station, 5 effects.

Figure 3 shows an excerpt from a sugar house model of a factory. With the AMS, sugar crystal losses occurring in a centrifugal can be easily detected. This information could lead to timely decisions on the need to replace faulty screens, for example, or indicate other problems within the sugar boiling station.

A fundamental problem regarding approximation to a true on-line balance is the different measuring intervals for various process data. Whereas, for temperatures, pressures and, for example, the cane mass flow values are usually recorded, e.g. every twenty seconds, the analytical data (dry substance, purities, etc.) is only available in an hourly rhythm or even much larger intervals. The on-line measurement of analytical data is, even today, not developed far enough as to measure all values precisely on-line. Apart from that, the costs for on-line measuring instruments today are still very high. The intervals for every measurement can be chosen freely with the AMS and thus be adapted easily to the factory system. The consideration of product retention times can have positive effects on the accuracy, as the non-sugar load brought into the process has a retention time of up to three days until discharged with the molasses.

Further advantages of the AMS/Sugars[TM] are: high flexibility, adjustment at any time or to new operating conditions and its simple installation and mode of operation. The specially designed Sugars[TM] model balancing and graphical displaying the factory operation can also be helpful to the management concerning decisions on capital investments. By means of variations of the Sugars[TM] model, many different proposals can be simulated providing an optimised result.
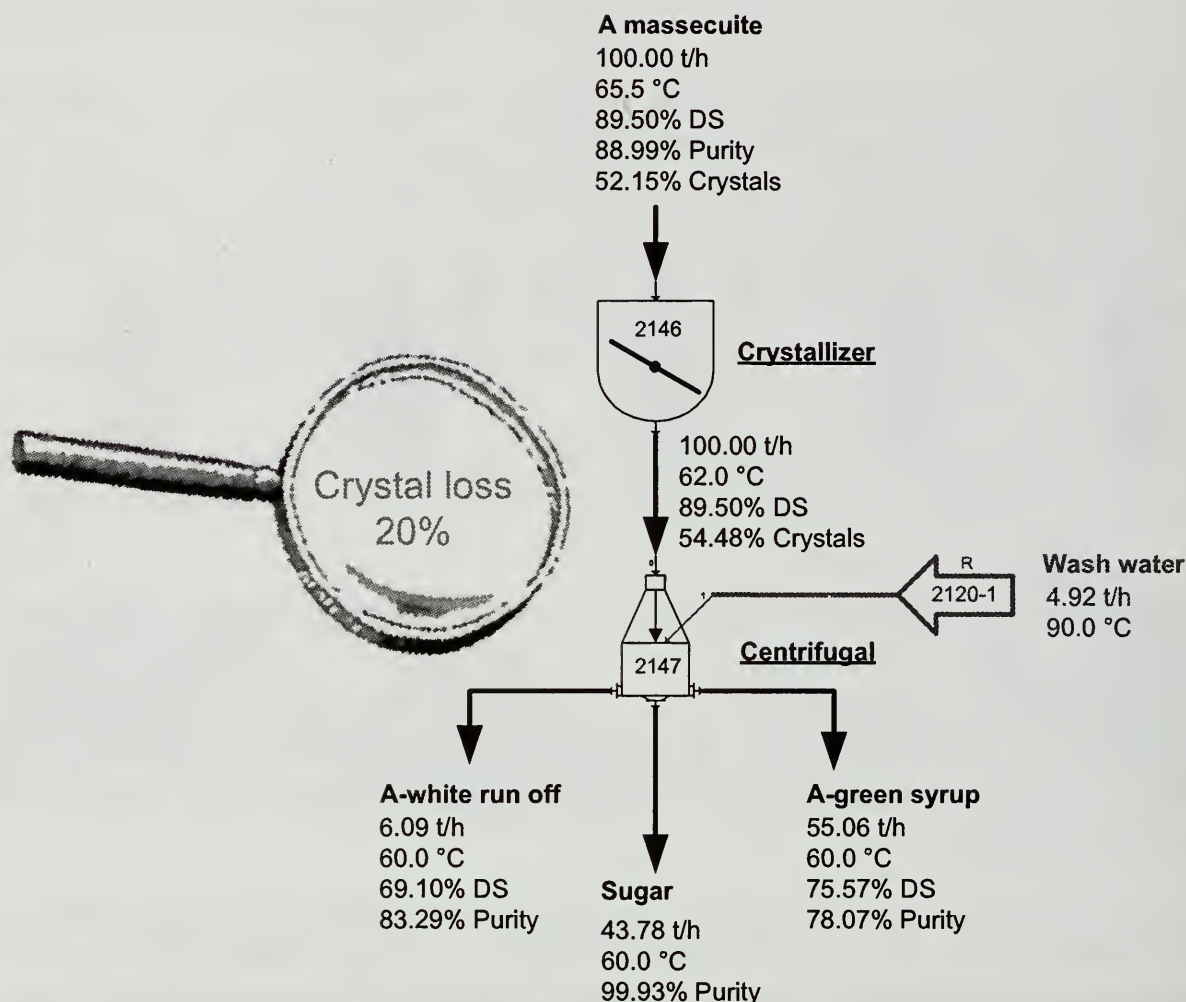
The XML-file with the actual process data can also be transmitted via Internet and thus allows the possibility of absentee diagnosis.

A massecuite
100.00 t/h
65.5 °C
89.50% DS
88.99% Purity
52.15% Crystals

2146   **Crystallizer**

Crystal loss
20%

100.00 t/h
62.0 °C
89.50% DS
54.48% Crystals

R
2120-1

Wash water
4.92 t/h
90.0 °C

2147   **Centrifugal**

A-white run off
6.09 t/h
60.0 °C
69.10% DS
83.29% Purity

Sugar
43.78 t/h
60.0 °C
99.93% Purity

A-green syrup
55.06 t/h
60.0 °C
75.57% DS
78.07% Purity

Figure 3. Excerpt from a sugar house simulation model.

First Experiences in Brazilian Cane Sugar Factories with the AMS

With the installation of an Advanced Monitoring System in three Brazilian cane sugar factories, first experiences have been made, and flawless function has been proven. The AMS offers the factory management a permanent mass and energy balance of the whole production process (transparent factory). Balance periods of 12 hr or 24 hr have proven to be reliable. A balance variant with process data collected at 15 minute intervals and the analytical data collected at a 12 hour interval also produces good results, but makes the system more sensitive to disruptions. The existing process data management system had to be adjusted to the demands of the AMS. The access to laboratory data whenever wanted - meaning a free choice of the interval for balancing - was not possible at first and had to be adjusted.

Outlook

Cutting down the data flood by introducing the AMS to only about 100 parameters needed for balancing the process allows, in the medium term, a significant reduction of measuring points in the PCS and in the laboratory, as virtually all operation relevant parameters can be calculated with the program. However, the demands on accuracy of the parameters used for balancing are high. Today, AMS data can be transferred directly into the data management system of the factory and calculated process values can be displayed in the factory PCS allowing the operating team an improved overview of the process. The "over-automation" sometimes observed in certain factories can be stopped and investment and maintenance costs can be lowered. Above all, we regard the AMS only as a necessary intermediate step for the implementation of expert systems.

Key performance indexes (KPI's) such as the k-values of heat exchangers and evaporators determined with the system could then be compared deliberately with benchmark data. Following this, for example, automatic cleaning orders could be given and a multitude of other functions are conceivable. Another important KPI is the centrifugal sugar crystal loss due to dissolving of crystals that can be monitored directly with the system.

Figure 4. Handling sequence of Key Performance Indexes (KPI's) of chosen equipment or process steps by means of the AMS.

Summary

With the Advanced Monitoring System (AMS), the whole production process of a cane sugar factory can be balanced and presented in a "transparent" simulation model. The AMS puts the operator in the position of fast recognition of disturbances in the process or of equipment and thus enables him to intervene at the right time and place. The AMS can also be the basis for a later installed expert system so that an automatic optimization of processes can go forward.
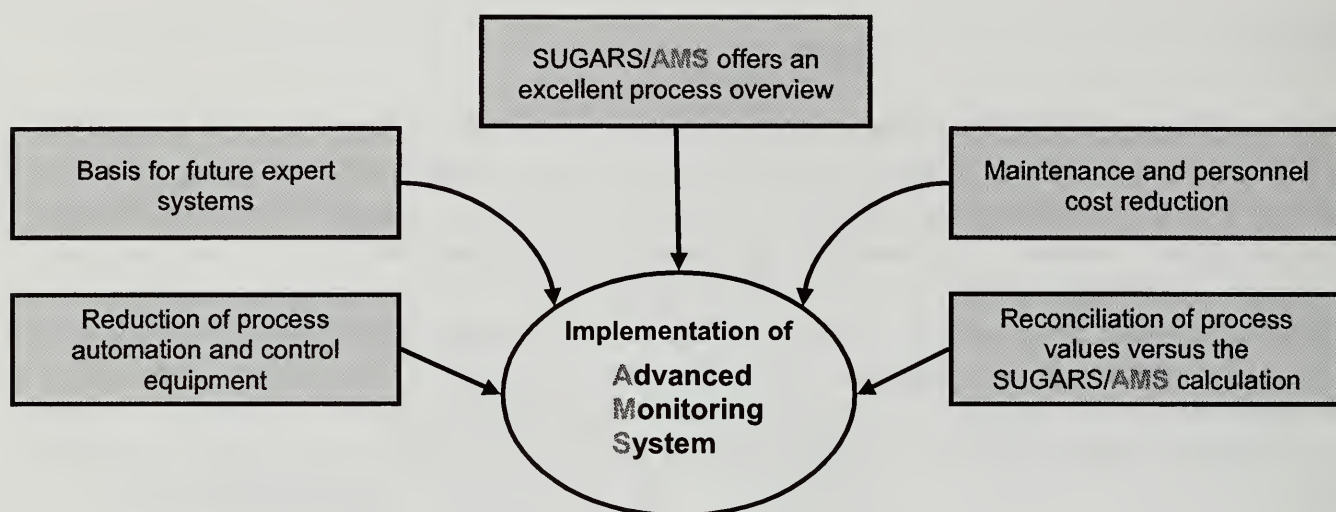
Figure 5. Decisive factors in favor of AMS implementation.

References

Morgenroth, B., Weiss, W., 2003: Advanced Monitoring Systems for process control, CITS in Madrid, 18 to 21 May 2003, Verlag Dr. Albert Bartens, 213-130.

Vogl, W., Koenig, H., Woehrle, K., Zahrer, R., 2000: Automatic integration of process and laboratory data into a Management Information System at the Südzucker factory in Offenau, International Sugar Journal 102, 522-529.

Pesticide Residue Analysis of Sugar

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Introduction

Necessity of Scanning Sugar

Sugar is one of the most frequently used processed agricultural products in the food industry across the globe. It is the key ingredient of a variety of food products consumed by all age groups. In recent years, with the advent of chemical pesticides being rampantly used to get agricultural products that are undamaged by pests, scanning all consumables for the presence of residual pesticides has become inevitable.

Sugar is a product of cane or sugar beet collected across vast agricultural expanses with little or no knowledge of Good Agricultural Practices (GAP). It is not just produced from a variety of lots of canes but also by multiple processing techniques (Plantation White Sugar, Refined Sugar, Raw Sugar, etc)

Its is also relatively unexplored in terms of exact chemical composition as regards level and nature of contamination, interference generated when scanned as a part of a finished product using chromatographic techniques. Hence it is necessary to scan sugar not just for the presence of pesticide residues but also for the kind of background generated when sugar is scanned over various chromatographic techniques.

Moreover, sugar is being subjected to the same kind of pressure as other food products as the consumer becomes more aware of the potential health hazards of various contaminants. It has already been subjected to scrutiny for heavy toxic metals and pathogenic microorganisms. As the food processes get more complex, the necessity of knowing each raw material in deeper aspects has become evident. Hence a thorough scan of sugar including all the physical and chemical parameters including pesticide residues is necessary before introducing it as a raw material in any food process.

This paper describes the procedure of validation and analysis of volatile pesticide residues in sugar using GC/MS technique.

Pesticide Residue Analysis of Sugar

List of Analytes

Sr. No.	Specific Test Performed	Sr. No.	Specific Test Performed
1.	2,4 DDT	19.	Butachlor
2.	4,4 DDT	20.	Alchlor
3.	2,4'DDD	21.	Atrazine
4.	4,4'DDD	22.	Methyl Parathion
5.	2,4' DDE	23.	Methyl Paraxon
6.	4,4;DDE	24.	Malathion
7.	α- HCH	25.	Malaxon
8.	β- HCH	26.	Aldrin
9.	δ- HCH	27.	Dieldrin
10.	γ- HCH	28.	Dichlorovos
11.	Endosulfan I	29.	Metribuzin
12.	Endosulfan II	30.	Carbofuran
13.	Endosulfan Sulfate	31.	Cypermethrin
14.	Monocrotophos	32.	Simazine
15.	Ethion	33.	Quinalphos
16.	Chlorpyrifos	34.	Methyl-Azinophos
17.	Phorate	35.	Ethoprophos
18.	Phorate Sulfone		

Equipment Used
Varian Saturn 2200 GC/MS system (With SIS and MS/MS^n)
Varian VacElut SPS 24 sample prep system
Temperature Regulated Water Bath

Materials and Chemicals
C18 cartridges (3mL) for SPE (Solid Phase Extraction)
Reagent water (HPLC grade)
Methanol (HPLC grade)
Ethyl Acetate (HPLC grade)
Nitrogen Gas
Sugar Test Reagent
HCL (0.1 N)
Sodium Sulfite (AR grade)

Method Used
The EPA method 525.2 which is used basically for analysis of pesticide residues in drinking water was validated for analysis of sugar /sugar syrup as a matrix.

Procedure

1. General procedure for Sample Extraction

One liter of 50% W/W solution of sugar was prepared in reagent water. The pH of the sample was adjusted to 2 pH units by the addition of 0.1 N HCl. Sodium sulfite was added to the sample.

The sample was then passed through a C18 cartridge preconditioned with methanol and reagent water, mounted over a vacuum manifold (VacElut SPS 24).

When the entire sample passed through the cartridge, it was desugarized with water until the exiting water showed no traces of sugar. The cartridge was then eluted with a total of 12 mL ethyl acetate to extract the non-polar volatile residues trapped inside the cartridge.

The extract was then concentrated to 1 mL volume on a 45^0C water bath and under a gentle stream of nitrogen.

2. Spiking of Analytes

Seven replicates, each 1-liter, of same sugar sample were prepared. Each replicate was spiked with 5 µL of 10-PPM solution of set A, Set B, Set C and Set D analytes. The resulting concentration of the desired analytes in the replicates was 0.1 µg/L.

The general procedure for extraction was followed for each replicate and each replicate was extracted under identical conditions.

Sevem replicate extracts were concentrated to 1mL each.

3. Calibrations and Analysis

Calibrations

35 different Organochlorine and Organophosphorous compounds were divided into 4 groups for ease of calibration.

A detailed SRM data was maintained for the generation of stock solutions, primary dilution standards, working solutions and calibration standards.

These groups were injected at 5 different concentrations ranging from 0.08 to 5.0 ppm.

This 5-point graph was used for the analysis of samples in replicates and the standard mixtures were used for intermediate QC checks.

Linearity Data

Calibration Curve Report

File: c:\coca-cola samples\set a msms 270606 modified.mth
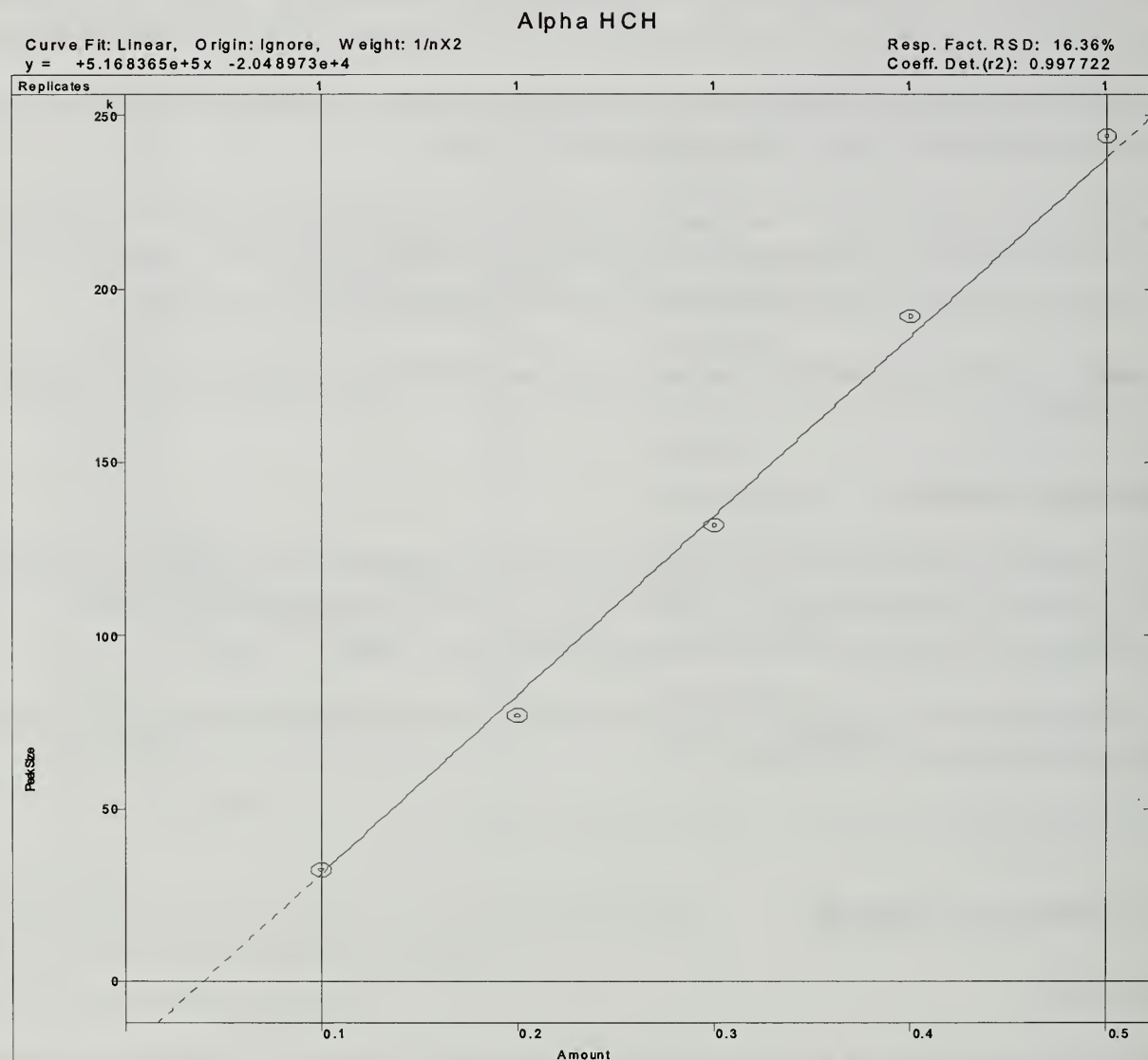Detector: 2000 Mass Spec, Address: 40

Figure 1. 5-point calibration curve of Alpha HCH

Analysis

The analysis was done on a GC/MS system. 1µL - 4 µL of each replicate was injected to the GC/MS depending upon the sensitivity of the analytes in question.

Chromatographic Conditions

Column: Varian Factor Four VF-5MS, 30m X 0.25 mm ID DF = 0.25
Temperature ramping: 45°C to 320°C in 39.67 minutes

SIS (Selective Ion Storage) ionization was used for all analytes, but for less sensitive analytes the MS/MS (fragmentation of parent ion) option was used and for co eluting peaks MRM (multi-residue monitoring) was also used.

The results obtained were analyzed for the following statistical parameters
- Relative Standard Deviation (RSD)
- Method Detection Limit (MDL)
- Mean Method Accuracy (as % recovery)
- Repeatability

Results

The results shown in Table 1 were obtained for all the 35 analytes.

Table 1.

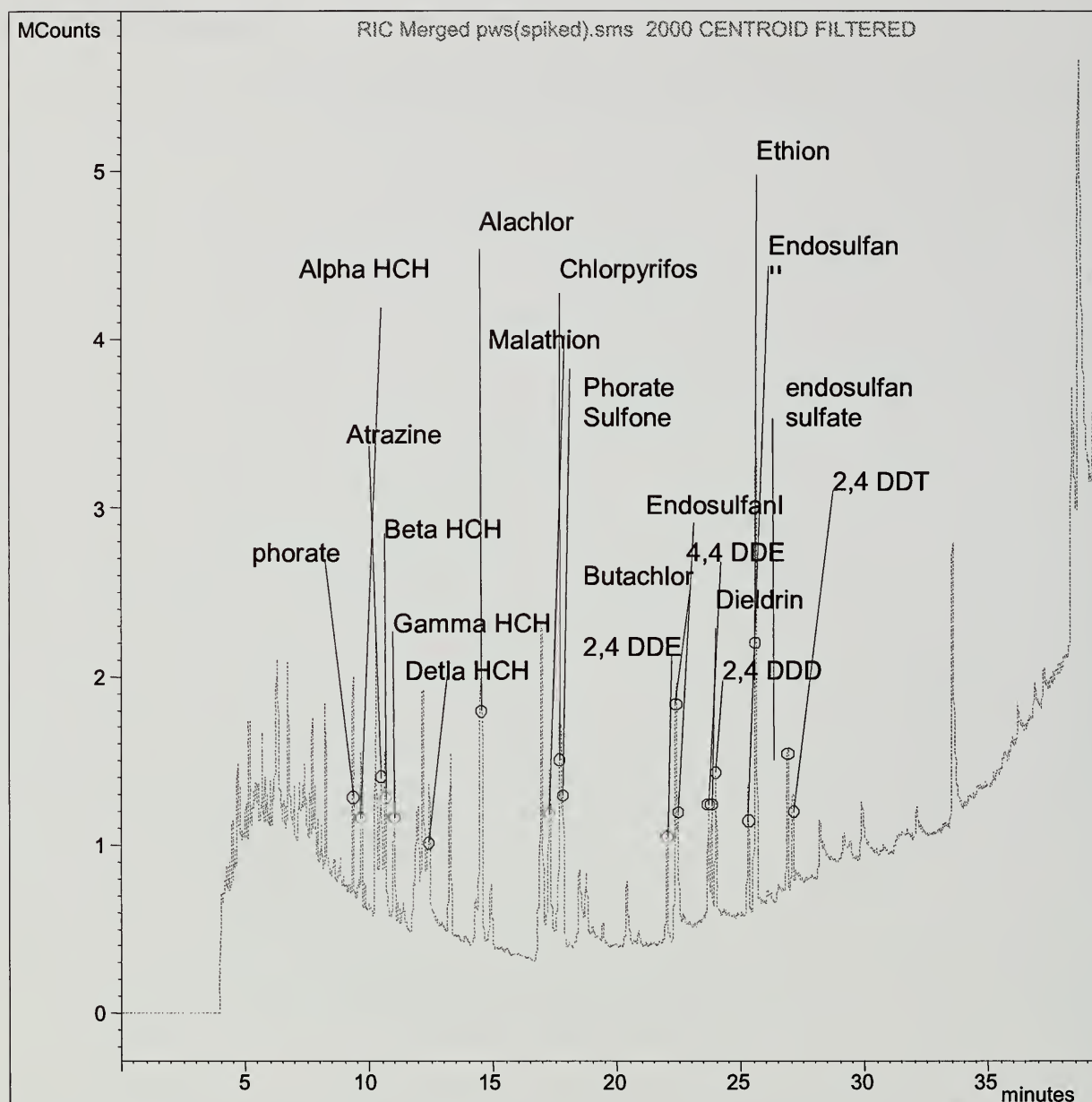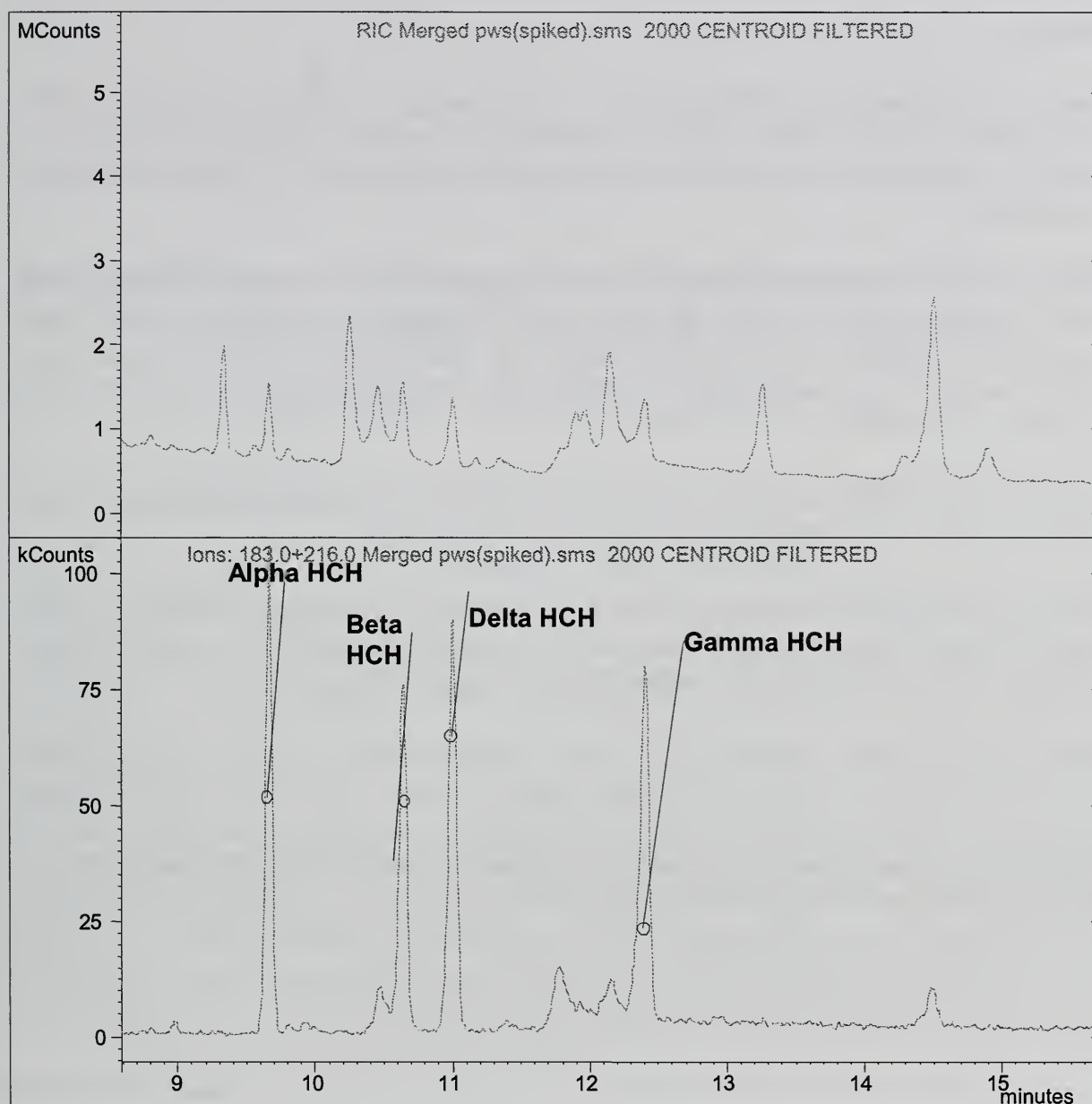| 1	Recovery	80 – 120 %
2	RSD	Less than 10%
3	Minimum Detection Limit (MDL)/LOQ	0.1µg/kg of sugar

Chromatograms
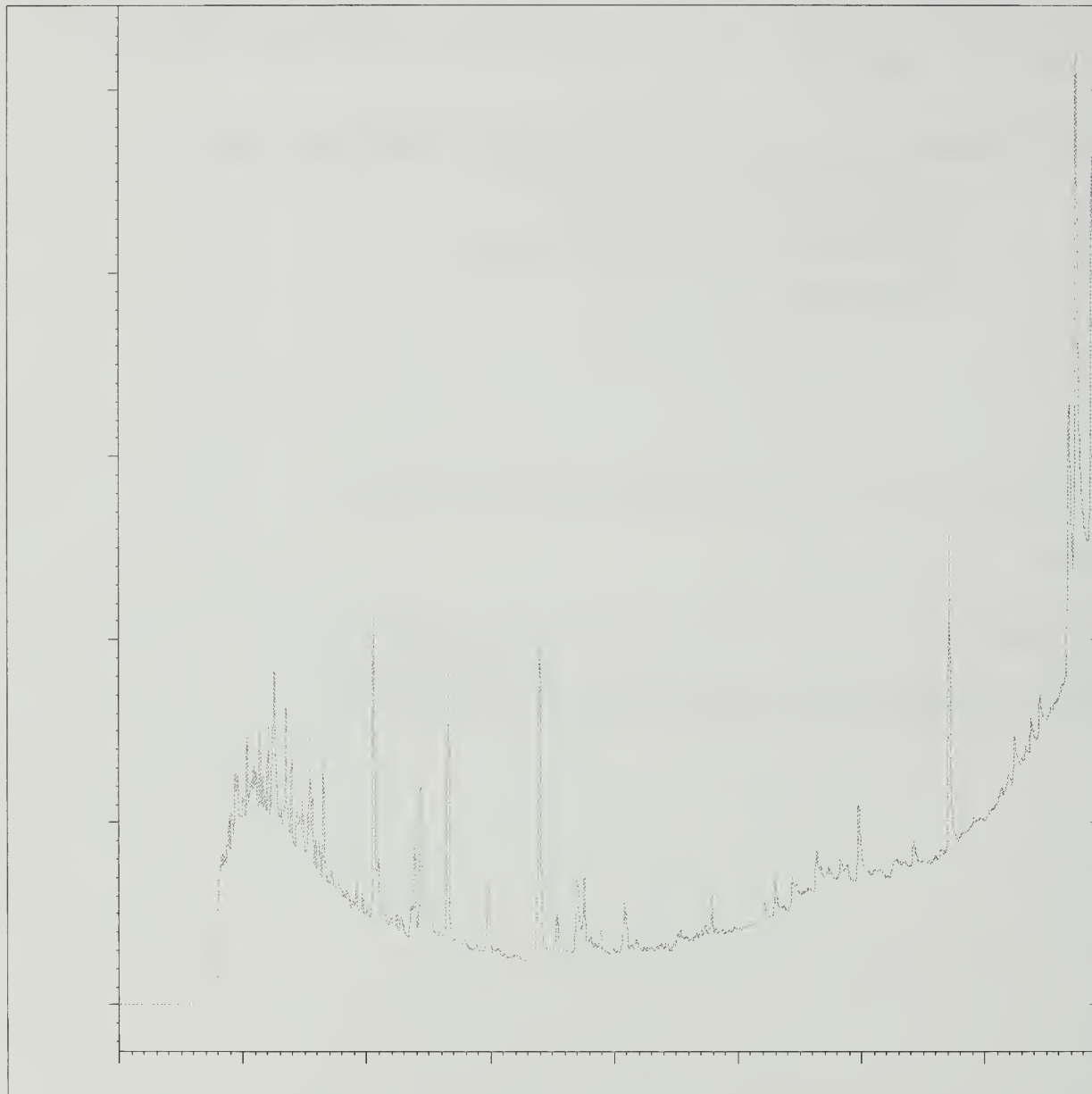
Chromatogram 1. Blank Plantation White Sugar.
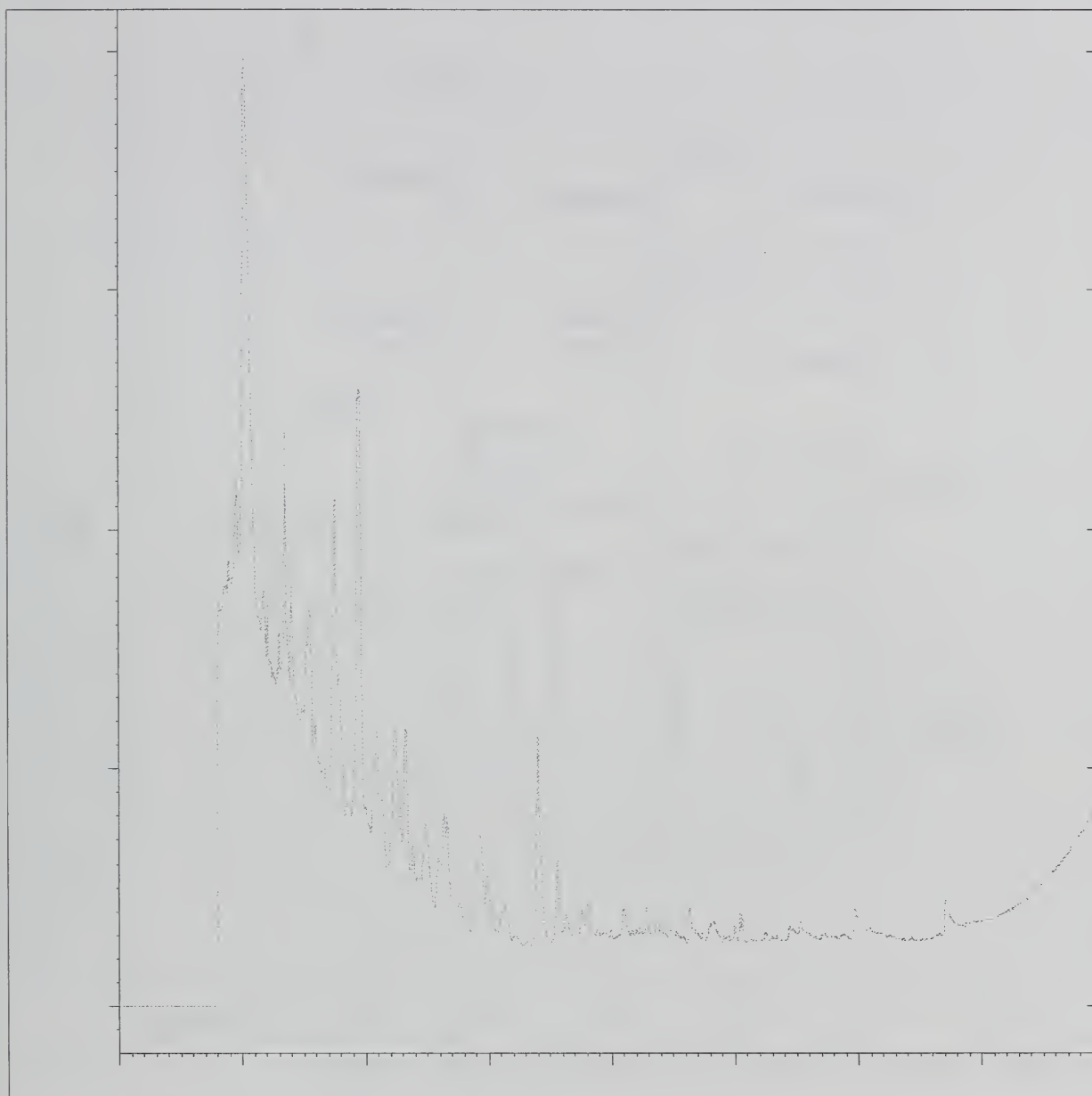
Chromatogram 2. Blank Refined Sugar.

Chromatogram 3. Plantation White Sugar spiked @ 0.1 μg/L analytes.

Chromatogram 4. HCH isomers separated from background using characteristic m/z 181 specific for HCH.

Conclusions

1. Sugar can be handled conveniently as a matrix using solid phase extraction (SPE). Using less solvent in SPE as against liquid-liquid extraction (LLE) helps improve the recovery, and reduces losses due to minimal use of glassware, solvents and manual operations.

2. The use of Mass Spectrometric detection greatly reduces the ambiguity associated with analysis of sugar. Sugar having a high background containing various plant derivatives, organic acids, phenolic compounds and sterols detection of trace level pesticides from such background is a formidable challenge in the absence of a mass detector.

3. The use of a mass detector greatly reduces the risk of misinterpretation of the background as a potential residual analyte.

4. The use of ionization techniques like SIS (Selected Ion Storage), MS/MS^n , MRM (Multi Residue Monitoring) helps resolve the problems associates with excessive background, final confirmation and co-eluting compounds respectively.

Maarc Labs Pvt Ltd. Is accredited for the analysis of pesticide residues in Sugar and Sugar syrup along with drinking water as per ISO-IEC 17025.

Bagacillo Removal Using Specially Designed Gyro - Rotary Vibroscreen to Improve Sugar Quality

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[2]Namokar Enterprises, New Delhi, India
[3]VM Biotech, 1004, Pune, India

Abstract

Modification in cane preparation devices, such as the shredder / fiberizor, have improved the preparation index (P.I.) and thus increased extraction at mills. However, it has increased bagacillo levels in mixed juice. Bagacillo releases coloring matter at high temperature, especially after liming. This rise in color due to bagacillo can be avoided or reduced by reducing bagacillo from mixed juice with the help of a proper screening device to remove bagacillo from mixed juice – the Vibroscreen. This device can be considered as a pre-clarification station and will help add value to the cane sugar produced by way of improvement in terms of ICUMSA color, keeping a low cost of production, and leading to greater profitability of the sugar industry.

Introduction

The main aim of the sugar process should be to avoid entry of the impurities rather than treating them later. This is also necessarily true about the coloring matter or precursors of coloring compounds through the basic input material that produces color during processing. All the techniques based on this fundamental principle of "**Prevention is better than cure**" do exert positive economical advantages.

There is good demand for sugar having low ICUMSA color, and especially in India, sparkling white sugar always commands a better price. With the improvements and induction of appropriate milling technology, extraction at mills has improved tremendously, but at the same time, there is a side effect, i.e. extraction of more fine bagacillo particles along with pith, which contains maximum coloring matter. Fine bagacillo and pith enter into the process, and their presence impacts the whole boiling house operation like growth and crystal shape, boiling time, etc. This also increases viscosity and affects sugar quality. There are several colloidal impurities, which enter through many inputs in the process, such as bagacillo in mixed juice; bagacillo and

pith in rotary vacuum filtrate; fine girt, sand, silica and undissolved particles in milk of lime and undissolved fine sugar crystals in melt.

Therefore, screening of the basic input materials, like mixed juice, filtrate, milk of lime, sugar melt, etc., through an efficient separation device is necessary.

Type of Colorants

In cane juice the following compounds are mainly responsible for original color and development of color during processing.

a) Plant pigments such as chlorophylls, xanthophylls, carotene and anthocyanins, etc., originally present in cane juice.

b) Some color production may take place during processing by exposure of juice to varying conditions like high pH, temperature and retention time. Mainly these reactions are caramelization, decomposition of sugars, destruction of reducing sugar, reaction of amino acid and reducing sugar.

c) Polyphenol compounds such as tannins, polyphenols, etc., which produce color during processing.

Impact of Bagacillo on Color During Processing

Bagacillo is made of fiber containing 50% cellulose and the rest comprising hemicellulose, pentosans, and lignins. These are high molecular weight compounds. Hemicellulose contains potassium while cellulose and lignin (component of the outer hard rind of the cane stalk) contains polyphenols. Polyphenols are originally colorless but on treatment with milk of lime and heating they become yellow and are extracted. Hence, it is essential to keep the quantity of bagacillo in mixed juice and in filtrate at a minimal level before it is treated with milk of lime. Polyphenols also react with iron and oxygen, particularly in alkaline solution, to form dark color compounds. The scalding effect on fine bagacillo having numerous open cells by the hot juice causes a molecular diffusion process resulting in 15% increase in turbidity. The concept of diffusion is further substantiated by an increase around 26% on P_2O_5 originally present in mixed juice. Turbidity is due to the very fine bagacillo, i.e. ionic or molecular dispersion. Bagacillo in juice is rich in P_2O_5, which is released on heating and causes more consumption of lime.

It is observed that an increase of 0.1% bagacillo in mixed juice increases clear juice color by about 2.5%, i.e. about 50-75 ICUMSA units.

Other Adverse Effects of Colloidal Impurities In Process

a) Higher lime & sulfur consumption.
b) Need of high capacity heat exchange vessels.
c) High pan boiling time.
d) Low crystal growth rate.
e) Poor color & grain size of sugar.
f) Poor exhaustion of sugar from molasses.
g) Low crushing rate.
h) Higher SPM, ash, color and invert levels in sugar.

Overall, the net result is increased cost of production.

Normal Practice

In an effort to reduce the fine bagacillo content in raw juice, before going to clarification, straining is done by a DSM SCREEN with 0.7 mm aperture. Later, double DSM screening with 0.7 mm and 0.5/0.3 mm opening in series were tried. However, many bagacillo particles are much smaller than 0.3 mm and can escape through the screen. It has been observed that the screens get choked quickly by particles close in size to the screen aperture, resulting in recirculation of juice on mills, thereby drastically reducing the efficiency of separation. Microbial sugar losses are increased since the choked screens act as a source of microbial growth.

In view of these persistent problems and to increase efficiency of separation, a rotary screen has also been tried as an alternative. But problems like jamming of holes, i.e. screen blinding by particles close in size to the screening aperture, overflow of juice along with bagacillo and huge water consumption for cleaning the holes were observed. Fine bagacillo continued to escape into the mixed juice.

This calls for a "SPECIAL DEVICE", which could effectively overcome the difficulties observed and to have the capacity for better separation such as:

1) Minimal screen blinding
2) Removal of suspended impurities and fine bagacillo
3) Minimum retention time
4) Minimum water, steam and power consumption.

This is possible if the screen is vibrating in three directions – with horizontal, vertical and tangential motion. Such moving screens will have the capacity to remove suspended impurities effectively without blocking juice flow.

The Device

VIBROSCREEN is equipment based on the technology of circular gyro-rotary vibrating screens having multi-plane three component mechanical vibrations which gives very high screening efficiency, and is thus a suitable device for the removal of bagacillo from mixed juice. The Vibroscreen is further modified to have all necessary requirements after conducting exhaustive trial for the various applications in sugar mills as stated below:

- Highest screening efficiency.

- Highest solid- liquid separation.

- High capacity per square feet of screen area.

- Long screen life.

- No transmitted vibration.

- Horizontal, vertical and tangential component of the motion imparted to the solid on the screen is controlled by top eccentric mass, bottom eccentric mass and angular position by top and bottom eccentric mass.

- Vertical vibration of the screen ensures minimal screen blinding by particles close in size to the screen aperture.

- Vibroscreen has low power requirement i.e. 1.5 H.P. (for 64" diameter) since almost the entire energy to the motor is used to create screen motion and nothing is wasted in vibrating a massive frame and foundation.

These Vibroscreens are available in sizes of 60", 72" and 84" diameter. Selection of screen mesh size is also equally important; higher mesh will reduce juice handling whereas lower mesh will not separate bagacillo efficiently. Thus the correct techno-economic balance is necessary. We have conducted various trials on a 60" model using 24, 40, 60, 80 and 100 mesh screens for various applications like milk of lime, filtrate, syrup and mixed juice. We found that for mixed juice use of 60 mesh screens removed more than 75% bagacillo with good juice handling. Table 1 gives data of several factories in removing bagacillo from mixed juice, and Table 2 provides differential data on bagacillo removal of varies sizes.

Factors Affecting Bagacillo Content and Its Separation:

1. Cane quality: Trash influences bagacillo content adversely, especially when it is dry.

2. Cane preparation: Setting of fiberizor / shredder, speed, crushing capacity influences bagacillo. Especially rise in P.I. from 82% to above 90% increases bagacillo content many times.

3. Milling: Speed of mill, setting, wear and tear of trash plate and mill can influence bagacillo.

4. Wax content: Wax in mixed juice is variety dependent and affects filtration and reduces capacity of screening / separation.

5. Juice temperature – the higher the temperature, the greater the filtration capacity.

6. Brix of mixed juice, the higher the brix, lesser is the separation capacity.

7. Solid – liquid ratio, more solids will reduce capacity.

Conclusion

The Circular Unitized Gyratory Vibroscreen can remove more than 75% of bagacillo from mixed juice, remove fine grit from milk of lime and suspended impurities from syrup. Thus it is the most appropriate technology for removal of impurities prior to clarification. It improves the efficiency of the manufacturing process, helping to produce sugar with low color, low ash and low SPM level. In sugar factories, due to various advantages like being simple to install, almost nil maintenance and low operating cost, Vibroscreens are being widely used for removal of impurities from mixed juice, rotary vaccum filtrate, sugar melt, sugar seed, effluent and clear juice.

Acknowledgement

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Table 1. Bagacillo removal at different installations.
60 mesh screen (0.27mm opening), 64" diameter

Factory		Bagacillo g/liter Inlet	Bagacillo g/liter Outlet	% reduction
5000 TCD	1	1.080	0.193	82.13
	2	1.067	0.190	82.19
	3	0.840	0.173	79.40
	4	0.640	0.173	72.96
	5	0.470	0.170	63.82
	6	2.500	0.515	79.40
7000 TCD	1	1.00	0.18	82.00
	2	1.36	0.37	72.79
	3	0.91	0.20	78.02
	4	1.37	0.45	67.15
	5	1.53	0.43	71.90
	6	1.14	0.34	70.18
	7	6.94	4.01	42.22
	8	1.44	0.44	69.44
10000 TCD	1	08.25	1.66	79.88
	2	08.53	1.81	78.78

Table 2. Bagacillo removal of different sizes.
80 mesh (0.195 mm opening), 60" diameter, diffuser juice temperature 79 ± 1^0C

Screen opening mm	Test 1		Test 2		Test 3		Test 4		Test 5	
	Inlet	outlet	Inlet	outlet	Inlet	outlet	Inlet	outlet	Inlet	outlet
+ 0.600	2.300	0.000	0.263	0.000	0.188	0.000	0.6875	0.000	0.0750	0.000
- 0.60 + 0.500	0.400	0.000	0.200	0.000	0.463	0.000	0.4500	0.000	0.1125	0.000
- 0.50 + 0.425	0.713	0.000	0.275	0.000	0.713	0.000	0.7325	0.000	0.3750	0.000
- 0.425 + 0.30	3.288	0.163	1.225	0.225	2.888	0.088	2.6250	0.100	1.875	0.1125
- 0.30 + 0.25	1.357	0.038	0.863	0.388	1.475	0.038	1.6875	0.0625	1.125	0.1500
- 0.25 + 0.21	2.250	0.513	0.875	0.350	1.825	0.450	2.2875	0.1875	1.275	0.1250
- 0.21 + 0.18	0.563	0.363	0.200	0.175	0.463	0.225	0.7000	0.0625	0.2375	0.150
- 0.18 + 0.15	1.538	1.450	0.650	0.500	1.213	0.975	1.1875	0.8375	0.6875	0.575
- 0.15 + 0.105	3.013	2.838	1.863	1.150	2.838	1.675	3.6125	2.4125	1.8125	1.7125
- 0.105 + .053	3.178	2.282	1.950	1.438	3.850	2.638	4.0875	3.0250	2.500	2.0375
Total	18.6	7.647	8.364	4.226	15.916	6.089	18.058	6.6875	9.444	4.8625
% Removal	58.89		49.47		61.74		62.97		48.51	

BIOSUGAR and BIOCLIN: A Natural Solution for Cane Juice Clarification and Disinfection

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Abstract

A new technology based on modified vegetable extracts is offered as an alternative for juice clarification with several other benefits in sugar processing such as biological control, sulfur elimination and massecuite viscosity decrease, allowing higher yield and productivity. BIOSUGAR's action mechanisms in color precursor elimination, bacterial action slowdown and complexation of metals are fully described. A new way for mill preventive disinfection is available with BIOCLIN. This sanitizing agent can keep mills free from gum-forming bacterial colonies by developing a protective layer over the surface of equipment that inhibits bacterial deposition, providing safe and environmentally friendly protection.

Introduction

SETA SA is a 67 year old company that supports reforestation and explores the manufacturing of tannin and its derivatives as bioflocculants for sugar manufacturing and other industry segments. SETA has three plants in Brazil and exports to more than 70 countries.

Sugar Juice Clarification

Current problems in Brazilian sugar mills:

In Brazil clarification requires sulfitation;
There are problems related to scale in heat exchangers and evaporators;
Residual sulfite in sugar;
Poor clarification when sugar cane quality is poor.

BIOSUGAR

BIOSUGAR is a multifuncitonal bioflocculant that was developed for clarification of sugarcane juice. It is produced from a modified aqueous vegetable extract of the bark of the wattle tree, black Acacia, or Acacia negra (*Acacia mearnsii* de wild). The wattle grows abundantly in Southern Brazil and is obtained from managed and certified renewable forests. Wattle has the additional advantage of being a leguminous plant that helps fix nitrogen in the soil.

Some Chemistry of Tannins

Tannins in general are found in the bark, fruit, leaves and heartwood of plants and are composed of polyphenolic compounds that react rapidly with protein and metals. Tannins bind to proteins to form insoluble or soluble complexes and also interact with many other organic compounds such polysaccharides.
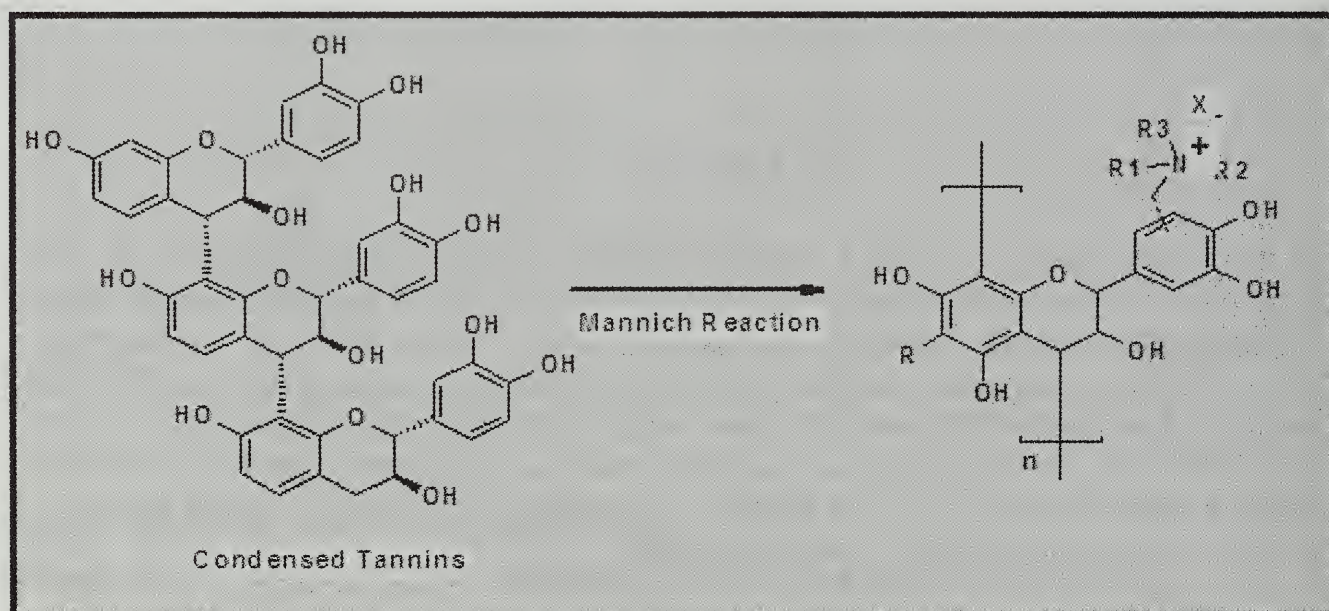

Figure 1.

Synthesis and Molecular Characterization

Tannin extracts are modified to contain a cationic charge and to increase their molecular weight. BIOSUGAR behaves like a medium molecular weight polymer and interacts synergistically with other flocculants, such as calcium phosphate and sulfate. BIOSUGAR is a cationic polymer with a strong flocculant action that interacts with organic compounds in sugarcane juice, promoting sedimentation. Due to ionic attraction and surface interaction, the impurities are rapidly eliminated by absorbtion and sedimentation, resulting in a clear juice.

The mechanisms of BIOSUGAR flocculant and sanitizing action effects on clarification include: Coagulating/flocculating action on organic and inorganic impurities; reaction with proteins to form insoluble complexes; strong chelating action with metals, especially iron; and biological control.

The interaction with polysaccharides is shown in Figure 2; e.g., the polyhydroxylated chain of BIOSUGAR interacting with starch.
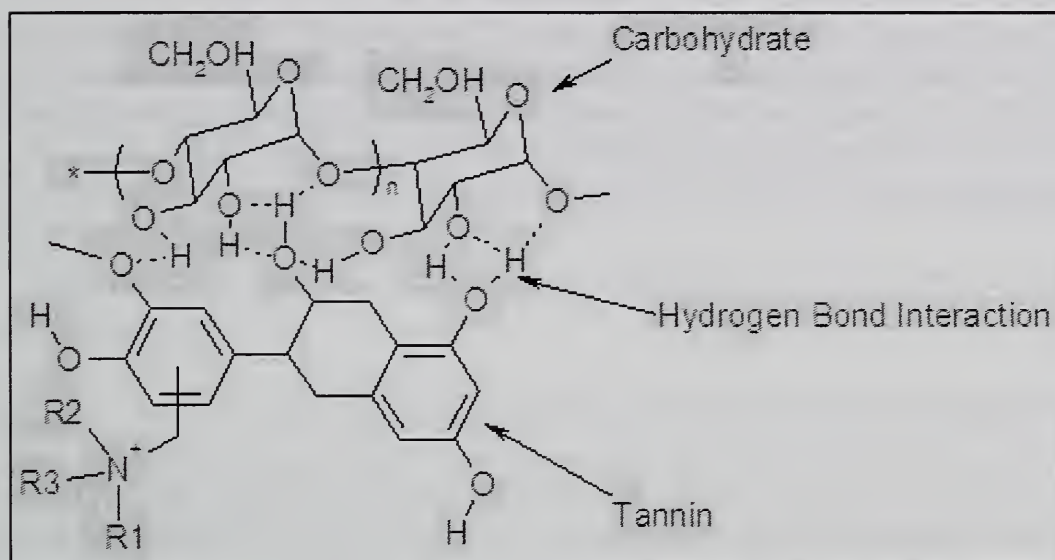

Figure 2.

Tannin based polymers present many hydroxyl groups in the right position with favorable chelating properties. An illustration of metal chelation is shown in Figure 3.
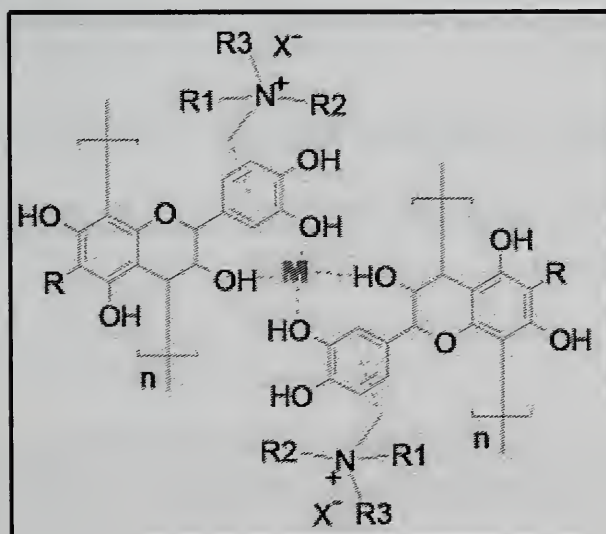

Figure 3.

BIOSUGAR is an excellent auxiliary flocculant for sugarcane juice, with a high sedimentation rate, higher juice flow, with less variation of clarified juice color. It allows better conditions of flocculation and sedimentation of impurities even when sugarcane quality is poor and there is deterioration and trash.

It allows for 30% to 50% reduction in sulfite use in the production of direct crystal sugar, and complete elimination of sulfitation in sugar production for refining. Reduction of lime consumption is around 10% to 15%. Because of the reduction of sulfitation, there is reduction or elimination of sulfite residues in the sugar produced.

There is less corrosion due to lower levels of sulfitation, and less scale in heat exchangers and evaporators.

There is reduction in the consumption of phosphoric acid and synthetic polymers.

In long shut-down periods, there is less inversion of sucrose in the clarifier, with less undersirable compounds such as dextran and organic acids forming because of the reduction of the juice bacteria population, once the cells are absorbed on the flocs and eliminated with the mud.

There is yield of higher density mud, which enhances the capacity of rotary vacuum filters, with lower losses in the filter cake.

BIOSUGAR acts as an antimicrobial agent in all the areas where treated juice is in contact, avoiding the formation and fixation of bacteria colonies in equipment, besides the anticorrosive action due to a protective film of iron tannate. Reduction of bacteria contamination also occurs in alcohol fermentation. There are also many benefits in fermentation due to a low level of residual sulfite. Sugar with better microbiological specifications is produced.

The product is easy to handle and dose, being a liquid that does not require dilution.

BIOSUGAR is a non-toxic and biodegradable product. The certification of tannates for use in food industries and production of organic sugar is already available.

BIOCLIN, Sanitizing Agent for Mills

BIOCLIN is a biopolymer also derived from vegetable tannin extract but with enhanced biological control mechanism. It is used to prevent microbial contamination through the inhibition of bacterial growth. BIOCLIN is biodegradable and nontoxic.

The slowdown bacterial growth is due to:

a) Interaction of tannin molecules with the polysaccharides in the bacteria cell wall membranes;

b) Chelating action probably reduces the availability of essentials metal ions for microorganism development (metallic ion privation);

c) Iron tannate coating over surfaces impedes the fixation of bacterial colonies.

BIOCLIN is applied dropwise on sugarcane conveyors, juice screens or cush-cush.

Advantages of BIOCLIN include: It keeps mills free of gum-forming bacterial colonies, helping to maintain the mills always clean; it diminishes the amount of synthetic biocides required; and it slows down / inhibits bacterial growth rate.

During the 2004/2005 growing season more than 2.6 millions of tons of sugar were produced by using BIOSUGAR and BIOCLIN.

Conclusion

In conclusion, it is emphasized again that these are products from Renewable Natural Sources. They represent Novel Technology as they are multifunctional products which act at several points on the production process of sugar and alcohol. They are biodegradable and environmentally friendly products with technical and economical advantages: They cause an overall improvement and stability in production process with safety and economy.

All Bioflocculants are obtained only from managed and renewable forests (FSC*). They are ecologically correct products and contribute to the fight against unauthorized clearing of jungle.

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*FSC: Forestal Stewardship Council.

Automated Method for Rapid Determination of Reducing Sugars

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In the sugar industry, determination of the reducing sugars can provide information about the quality of the raw material, process control and characteristics of the produced sugar. The Lane-Eynon method is often used for this determination. This method is very accurate if all the procedures are performed with much care and by experienced analysts. Its main drawbacks are the long time delay, complicated procedure, and heavy reliance on experience. In this paper an automatic method based on flow phototitration is proposed, which has been widely used for routine analysis in the sugar industries in China.

In this system, two linear-flow piston pumps carry the reagents (Fehling working solution A and B and titrant) to a titration cell with a precision of 2 µl. The titration cell, made of modified polyester (black), has a volume of about 10 ml. A temperature sensor and a heating bar are inserted into the titration cell. A light-emitting diode (575-590 nm) and a phototransistor, opposite to the LED at a distance of 15 mm are mounted in the cylindrical wall of the cell. A porous tube is placed along the light path, and a magnetic stirrer is placed on the bottom of the cell. A single-chip microcomputer is used for pumps, temperature control, data acquisition and processing.

The procedure is programmed under the control of the microcomputer. Two heating steps are provided for quickly increasing and maintaining a higher temperature for the reaction solution. At first, when Fehling solutions A and B are delivered to pass through the tube near the wall of the cell, the solution is heated by a heating coil and reaches a temperature of about 50 - 55° in 35 sec. The next heating step is performed by a heating bar and the temperature of solution in the cell can be increased to 98-100° in 25 sec and maintained between 98- 100° during the titration process. The end-point is determined by a sharp voltage change, and the titrant volume is recorded and the concentration of reducing sugar is calculated automatically. The result is shown on the screen and printed at the same time.

For reducing sugar determination, the bubble interference formed during boiling was eliminated by the porous tube along the light path. Glucose solution of 0.35% was used as the titrant and the injected sample volume was 200 µl. The linear concentration range for reducing sugar was from 0 - 1.0%. The reagent consumption was 7 ml. The photosensor response range was 0.1 - 1.8 mV. The determination time was less than 3 min. The results show that the relative standard deviation was 1.5% (n = 8) and had a good agreement (r =0.9999) between the present method and the conventional method. There was no interference formed by heating bubbles and the turbidity of the samples. This method is simple and rapid, has low reagent consumption with excellent accuracy and precision, and was suitable for routine analysis.

DRD – Dedini Refinado Direto (Dedini Direct Refined) – The Refined Sugar Without the Refinery

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Abstract

Production of granulated refined sugar from sugarcane has shown little evolution in the last years in Brazil. This paper describes the new process of fabrication of refined sugar, DRD-Dedini Refinado Direto (Dedini Direct Refined), that allows the production of refined sugar directly from sugarcane juice, with only one crystallization - and compares it to the conventional process. Tests conducted in sugar mills in Brazil, the results obtained, and the advantages of the new process are presented.

Introduction

Technologies for the production of raw or refined (white) sugar have not evolved significantly in the last decades. In fact, some minor improvements have been made, but the last great process developments were the continuous vacuum pan– during the 70s – applied to the sugar mill, and the use of resins – in the 80s – used in the refinery.

This paper presents what we understand is the next technological revolution in the production of refined sugar from sugarcane: DRD-Dedini Refinado Direto (Dedini Direct Refined).

The traditional solution to obtain refined sugar takes two big stages: the production of raw sugar in sugar mills, and the refining process in a refinery. In general and brief lines, juice is obtained from sugarcane and is purified and concentrated in sequential stages, resulting in a syrup that also passes through purification, then is crystallized to produce raw sugar. Raw sugar is then delivered to the refinery, where it is dissolved in water, producing a liquor that is also purified and concentrated in several stages and, finally, white sugar is obtained by crystallization.

A critical evaluation of this process leads to the conclusion that in the two stages the same kind of operations are performed, although using different techniques. But in essence, sugar dissolved in water (in juice or in liquor) is repeatedly purified, concentrated and, finally, crystallized. So, the process requires investments in the mill and in the refinery, the use of a large amount of equipment, and twice as much energy consumption in evaporation and vacuum, and in crystallization. Therefore, why not develop a new process where the stages of purification are more efficient, so that refined sugar is obtained directly from juice, bringing down costs and investments and with less energy consumption? For this purpose the development of DRD was initiated to obtain white sugar with one step of crystallization only, and at the sugar mill.

The Traditional Production Process of Granulated Refined Sugar from Sugarcane

In the conventional fabrication process of granulated refined sugar, the initial feedstock is raw sugar in its most diverse types(*): white crystal, VHP, demerara, etc. and its production process can be seen in the first block (the "sugar mill" block) of Figure 6. The raw sugar is dissolved in one or more equipment designed for this purpose, called melters. In the melter, sugar is diluted with heated water and submitted to stirring to obtain the liquor of dissolved sugar in the desired concentration. The liquor is then pumped to a filtration or screening system to eliminate rough impurities and then is sent to decolorization. Such decolorization can be effected by different processes: phosflotation, ion exchange and use of granular activated carbon, or an association of the same, depending on the quality of feedstock and the desired end product.

In the phosflotation process, phosphoric acid is added to the sugar liquor and then neutralized with hydrated lime. In addition, decolorizing and flocculating agents are added. Next, liquor is heated in a heat exchanger and aerated in appropriate equipment, being then sent to flotation where is separated in a light phase comprised of flocs that retain impurities and a denser phase that is the flotated liquor. Such flotated liquor is filtered in sand filters and can be sent to complementary decolorization by ion exchange resins or carbon filters and then sent to sugar crystallization and drying.

In the crystallization sector, clarified liquor is concentrated in boiling vacuum pans up to the supersaturation point. Next, an amount of pulverized sugar is added, which acts as seed for boiling. After this stage, sugar boiling is conducted up to the point where sugar crystals reach the right size and the boiling vacuum pan is completely full. Next, the same is discharged in tanks called crystallizers and sent to centrifugation in equipment called centrifuges, where crystals of sucrose and surrounding molasses are separated by centrifugal force. Sucrose crystals are then sent to drying and packing and molasses returns to the process up to the exhausting point.

Such sugar is then sent to a dryer where humidity is eliminated up to 0.03% - 0.05% by contact with heated air and cooled to about 40°C for packing and storage. If produced sugar will be stored for a long period of time, silos specially designed for conditioning are necessary. Sugar is then packed in bags of 1, 2, 5 or 50 kg, or big bags of 1200 kg, according to the market for which it is destined.

(*) Diverse types, example: white crystal or crystal with ICUMSA color of 100-200 and 99,6 – 99,8 pol; VHP (Very High Polarization) with ICUMSA color of 900-1200 and 92-93 pol; demerara (crude sugar) with 2000-3000 ICUMSA color and 90-91 pol.

DRD Process Description

In this new production process of refined granulated refined (white) sugar, the same is obtained from sugar syrup without the need of sugar melting and recrystallization.

During sugarcane processing, juice rich in sucrose and concentrated in 13-17% of total soluble solids are extracted. After being heated, such juice is clarified in a sulfo-defecation process using the NEW DEDINI SULPHITATION system and clarified in the NEW DEDINI JUICE CLARIFIER, which are equipment with higher efficiency, performance and reliability than the conventional equipment currently available in the market.

After the clarification process, juice is sent to multiple-effects evaporators, including the NEW DEDINI FALLING FILM EVAPORATOR, where concentration is increased up to 60-65 Brix (percentage of soluble solids). This concentrated juice is called sugar syrup.

Syrup, after leaving the last evaporation stage is sent to flotation in the NEW DEDINI SYRUP FLOTATOR, where phosphoric acid, a cationic decolorizing agent, calcium saccharate and floccing agents are used. After leaving the flotator, the flotated syrup is sent to a NEW DEDINI SYRUP FILTER which uses centrifugal force to separate impurities. The filtered syrup is then sent to the NEW DEDINI DEEP-BED FILTERS, which are use special sand for filtration, and then sent to the NEW DECOLORIZING SYSTEM by ion exchange. This system uses ion-exchange columns where synthetic resins specially designed for syrup decolorization are used. This decolorization system comprises three phases: (1) syrup softening; (2) decolorization; and (3) polishing or complementary decolorization.

Such softened and decolorized syrup is then sent to sugar crystallization where it will be used in the NEW DEDINI BOILING SYSTEM. This system is based on the principle that the highest solution purity be pursued in the solution that will be the feedstock for boiling, from which sugar to be packed will be delivered. For this purpose, besides the equipment usually existing in the plant, additional equipment, such as continuous centrifuge, crystallizers, dissolver and process pump are used.

After boiling, crystallization and centrifugation, sugar is sent to the dryer, and then to packing and storing, as described previously.

Figure 1 shows a block diagram of the DRD process, comprised of three modules, which contain the new projects of equipment or processes developed by Dedini to improve sugar quality. Existing facilities can also be adapted to perform the DRD process, as mentioned in the following diagram

The sulphitation stage can be replaced by carbonation with the required adaptations, maintaining the other stages of DRD.

Figure 2 presents the three modules of DRD process, and also emphasizes that the existing facilities should be analyzed, once they may be used for DRD, without the need of new equipment, on a case by case decision.
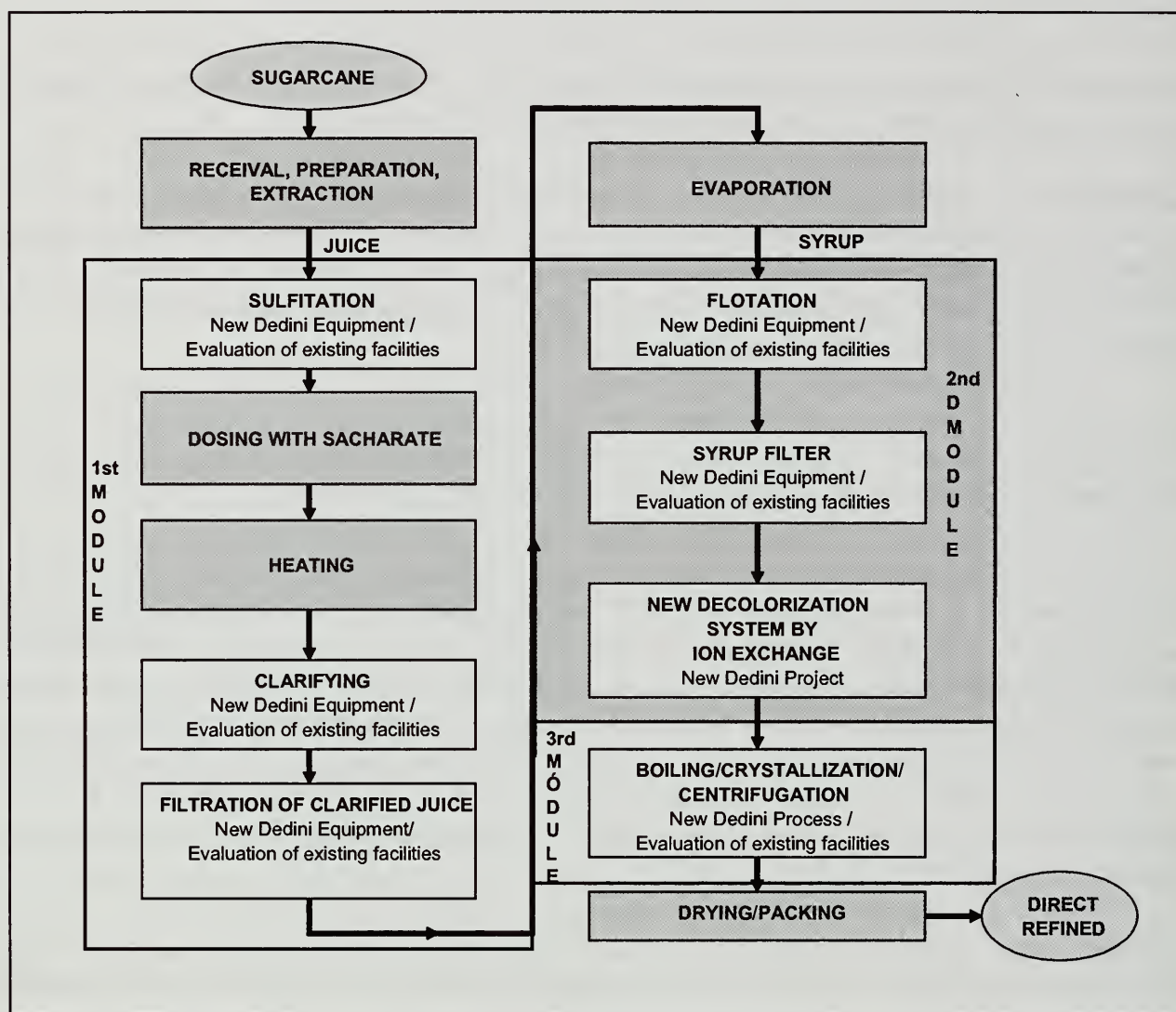
Figure 1. The DRD – Dedini Refinado Direto (Dedini Direct Refined) - Process

DRD Experimental Tests

When Dedini developed the DRD process, several pilot plants were conceived and built, being the main one among them the pilot plant for syrup decolorization by ion exchange. In this pilot plant, decolorization tests have been conducted in conventional sugar factories existing in mills located in the state of São Paulo, Brazil. The main objectives of the experimental tests were:

- Determining the efficiency of resins in decolorization of solutions with high concentration of coloring matters (syrup).
- Evaluating the useful life of resins for the application.
- Determining the technology technical-economic feasibility.

The project started in late 2003 through a joint venture between Dedini and one of the biggest resin manufacturers in the world, Rohm and Haas. The first stage comprised the construction and installation of a decolorization pilot plant in a mill, which operated during the 2004 and 2005 seasons under the most diverse industrial conditions.

FOR EXISTING FACITILIES, DIFFERENT OPTIONS MAY BE USED

MIXED JUICE

1ST MODULE
JUICE
CLARIFICATION

DEPENDING ON THE EXISTING PLANT AND QUALITY OF THE CLARIFIED JUICE OBTAINED, THE EXISTING FACILITIES MAY BE TOTALLY OR PARTIALLY USED.

CLARIFIED JUICE

2ND MODULE
SYRUP
DECOLORIZATION

NEW PLANT

SYRUP

3RD MODULE
BOILING/
CRYSTALLIZATION/
CENTRIFUGATION
SYSTEM

THE EXISTING EQUIPMENT MAY BE TOTALLY OR PARTIALLY USED, PROVIDED THAT THEY MEET THE DESIGN CONDITIONS AND, WHEN NECESSARY, ADDITIONAL EQUIPMENT ARE ADDED.
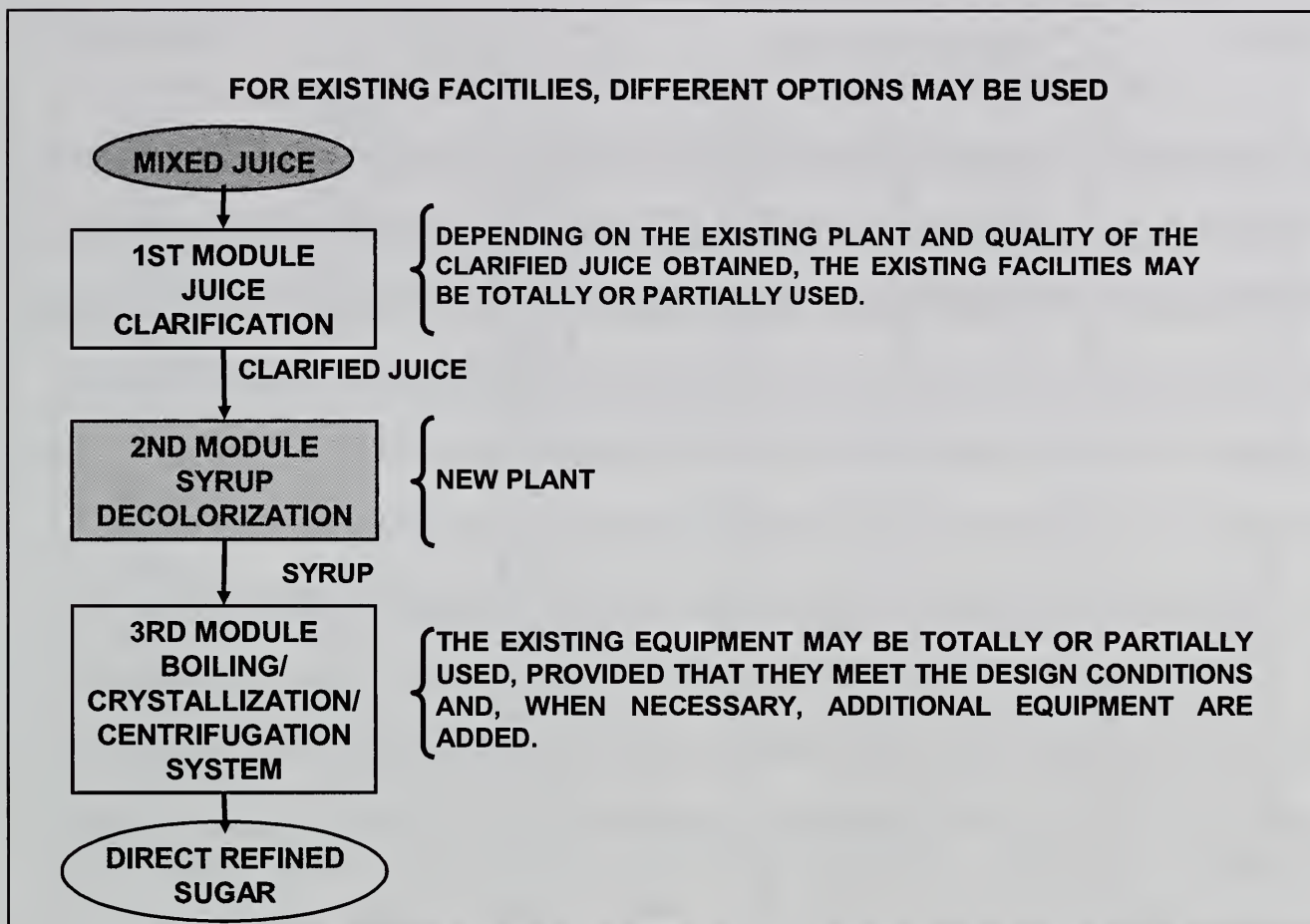
DIRECT REFINED
SUGAR

Figure 2. Fitting existing installations to process DRD.

Tests were conducted by operating the plant in cycles of approximately 30 continued hours utilizing the same resin. Figure 3 shows some of the results of the decolorization process in 20 selected cycles of the tests. Figure 4 shows the results of syrup decolorization by ion exchange.

After decolorization, syrup was crystallized and centrifuged, and some of the results of crystallization are shown in Figure 5.

At the end of the decolorization tests, samples of the resins used were analyzed in the laboratories of Rohm and Haas in the United States regarding its applicability in the process and life cycle. The conclusion was that the results reached the objectives.

Comparative Results and Advantages: Traditional Process x DRD

Figure 6 compares the existing stages of the conventional process and the required stages for the new DRD process.

Cycles	Color removal (%)			ICUMSA Color		Volume
	1st Stage	2nd Stage	Total	Input	Output	used (L)
1	21	39	55	8,315	3,800	3,654
2	14	35	44	6,852	3,693	5,460
3	25	36	45	8,776	4,024	5,472
4	27	31	51	8,042	3,729	5,480
5	22	32	56	8,387	3,596	5,470
6	21	18	30	9,753	6,096	10,032
7	27	35	56	9,780	4,040	5,016
8	24	33	55	7,527	3,600	4,560
9	33	33	56	7,784	3,296	4,104
10	39	44	70	6,800	2,100	3,192
11	25	23	42	7,420	4,160	7,296
12	20	48	64	7,600	2,740	5,016
13	33	23	55	7,958	3,476	5,481
14	26	37	54	7,720	3,593	5,474
15	34	27	53	7,515	3,555	5,928
16	23	29	51	8,420	4,220	5,918
17	35	39	62	9,667	3,744	5,016
18	26	41	62	9,820	5,633	6,842
19	26	34	56	8,180	3,622	3,192
20	23	29	52	8,360	4,231	3.650
Average	26.2	33.3	53.5	8,234	3,847	106,253

Figure 3 (above). Efficiency of syrup color removal.
Figure 4 (below). Graph of efficiency of syrup color removal.
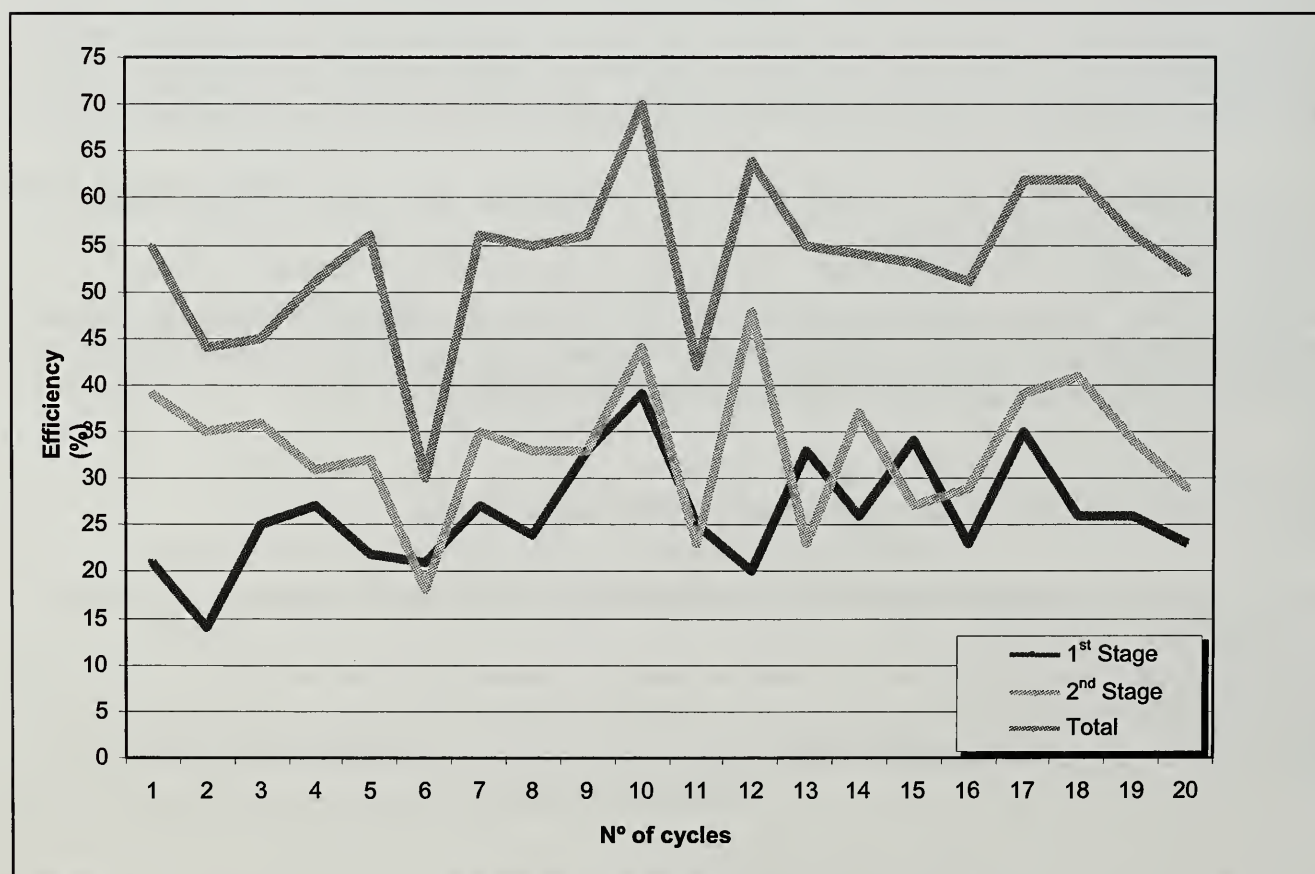
Crystallization	Average syrup ICUMSA color	Average sugar ICUMSA color
1	3643	37
2	4291	52 (*)
3	4557	62 (*)
4	5710	32
5	5237	44
6	5389	42
Average	4804	44

(*) deviation in operational standards parameters

Figure 5. Results of ICUMSA color obtained after crystallization with a 200 hl vacuum pan in Brazilian sugar mill
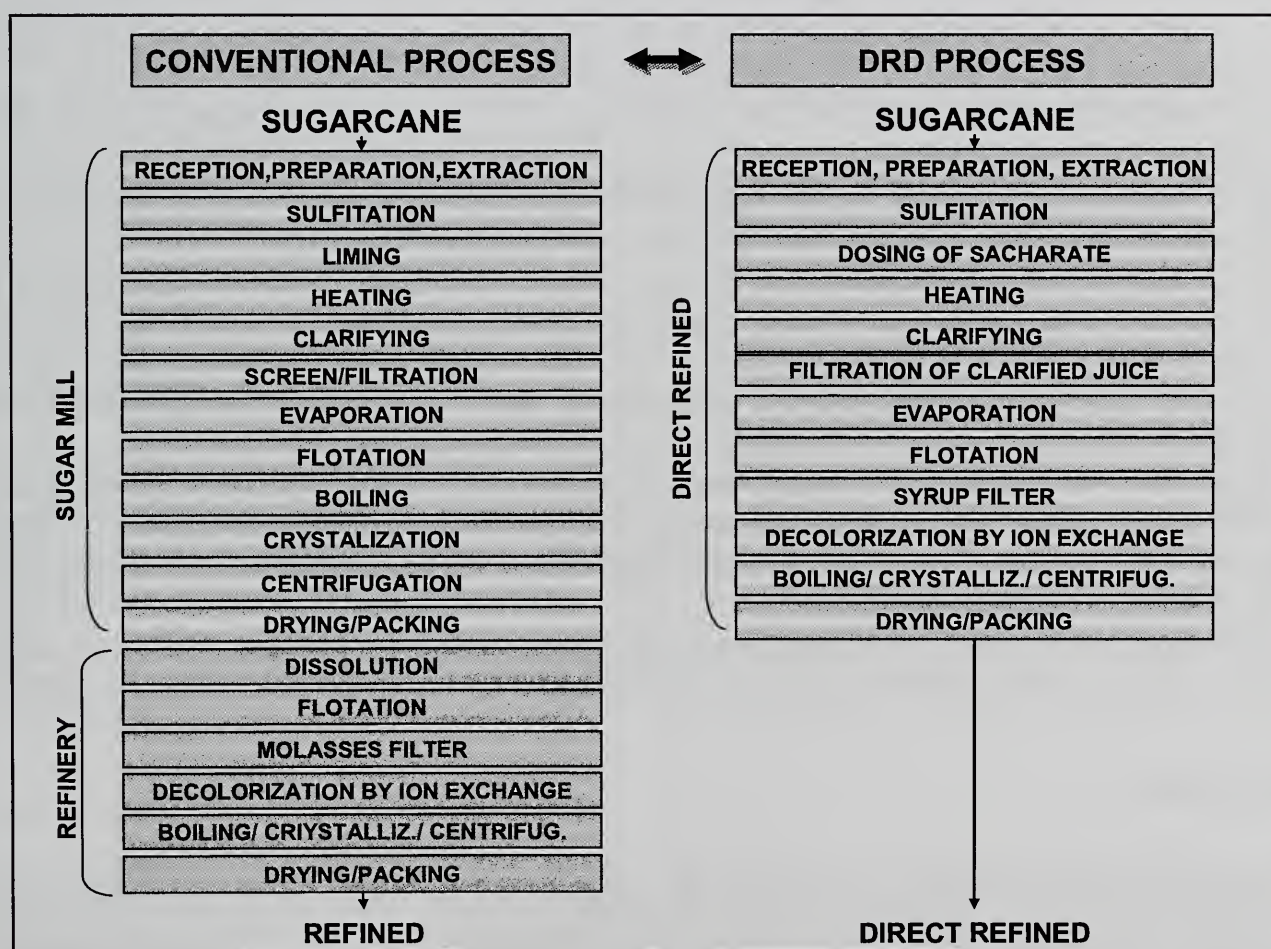

Figure 6. Comparative: traditional process x DRD process

Advantages of the DRD Process When Compared to the Conventional Process

The use of DRD has a number of advantages over the traditional production system:

- Less steam consumption in the fabrication process due to several energy demanding stages that are eliminated, mainly sugar melting and boiling.

- Less consumption of electrical power when compared to a refinery attached to a sugar factory: 7.5 kW/bag of 50 kg in the conventional process versus 5.0 in the DRD process.
- Less cooling water and process water required: In the conventional process, assuming the use of water multi-jets in vacuums plus, water needed for sugar dilution and de-sweetening/cleaning, approximately 7.2 m^3/h per bag of 50 kg of sugar produced; in the DRD process, we have 3.7.
- Less labor required: reduction of 40%, without taking into account labor for sugar packing (at the end of the mill process), and unpacking (before the melting stage).
- More compact installations in one plant only, thus eliminating investment in building and additional facilities for the refinery equipment.
- It can be supplied as a complete, fully new plant, or in separate modules, according to the mill's need. Besides, it permits that the existing plants and equipment be reused, thus reducing the initial required investment.
- It eliminates the need of handling the crystal/VHP sugar already packed/stored, or in bulk, thus eliminating losses of handling raw material as it occurs in the conventional process, besides the cost in the production process.
- Possibility of supplying not only equipment, but an integrated solution to meet the customer specific needs.
- Fabrication costs in the Brazilian conditions are about 25% lower, when compared to the traditional process, without taking into account reduction of losses.
- It allows the implementation of flexible plants, which may produce VHP, crystal and refined sugar in the same unit, conferring more operational, financial and marketing flexibility. In this case, the existing facilities are analyzed and evaluated, the equipment with adequate performance is maintained, and the plant is complemented with installations and equipment that are needed for the production of refined sugar.
- It gives as additional advantage the assurance of obtaining a constant quality product throughout the season, by eliminating the influence of long rainfall periods, feedstock quality variances, etc. This guarantees flexibility on trading contracts and production schedule with assured efficiency.
- Together with this new process, Dedini developed with own technology and now offers a wide line of equipment dimensioned and designed for higher efficiency and productivity.

Investment

When comparing the required investment for implementing a sugar factory to produce 10,000 bags/day it will depends of the specific conditions where the new plant will be installed. In order to compare the relative investments, we have the following situation to Brazilian conditions, shown in Figure 7
.

The DRD process was commercially available in the first semester of 2006. Due to its cheaper solutions and other advantages as described above, DRD is being introduced into the market, exactly at a crucial moment for the sugar producers: with the recent decision of WTO (World Trade Organization) that determined the gradual elimination of the subsidies on white sugar exported by the European Union, based on claims filed by Brazil, Australia and Thailand, an opportunity for exporting about 4.5 million tons of white sugar is open, and the most competitive

mills may occupy (and we believe it will do) a big portion of this market share; the implementation of DRD will contribute to this objective.

Type of plant	Investment (%)
Traditional factory (VHP and crystal sugar process)	100
Refinery (dedicated)	140
Traditional factory with refinery (traditional, integrated)	180
DRD factory (raw and refined sugar)	130

Included: Equipment for a complete turnkey plant with juice treatment, evaporation, vacuum pans, decolorization, crystallization, centrifugation, sugar drying, etc, interconnections, basic automation, assembly, start-up, commissioning.

Not included: civil works, buildings, complete automation, support services (laboratory, workshops, offices) and sugar warehouse.

For the existing units, investment will be determined on a case by case basis.

Figure 7. Comparative investments.

Impacts of DRD Process

The DRD process has significant impact on the sugar and alcohol industry. It allows production of a higher value-added product by the mills, with expressive commercial and financial reflexes. The mill is then capable of producing lower cost refined sugar and, at the same time, maintaining its capacity of supplying raw sugar, crystal or VHP. Such flexibility in production permits that the mills serve "just in time" – minimizing storage of end products – the best sale condition in the short term, according to the market demand at the time. The most important impacts of DRD are: technological, commercial and economic.

Technological

We understand that DRD represents a big leap in the evolution of the production process of refined sugar, and consequently reaches and starts a higher technological degree. From this higher level new advances should arise, as well as new processes that will represent improvements in a new cycle of development. DRD process and equipment is patented in various countries on all continents.

Commercial

The cost of DRD refined sugar is lower than those produced by the traditional processes.

For this reason we see commercial impacts of three natures:

a) DRD white sugar can replace a portion of the current demand for the traditional refined sugar;
b) New suppliers will take part in the refined market: the sugar mills that will be equipped with the DRD process. Such mills will pass from raw sugar suppliers to white sugar suppliers, which will replace the product of the refineries. The mills that currently export raw sugar will export refined sugar at lower prices, competing directly with the refineries that import raw sugar;
c) Lower cost allows the market to offer the product at lower prices. So, demands that today are repressed by the higher price of the refined sugar may be fulfilled by DRD: consumers that use today raw sugar for direct consumption will prefer DRD refined sugar sold at higher prices than raw sugar, but lower than the traditional refined one.
d) Finally, it must be emphasized that an increasing pressure is being imposed by the international trade for reduction of subsidies. As a consequence, opportunities will arise for new agents to fill the spaces that will be opened. Therefore, by implementing the DRD process, the most competitive countries and producers of raw sugar will tend to conquer significant market shares for refined sugar.

Economic

The economic result of the introduction of DRD process derives from two different origins:
- the cost of DRD refined is lower than the traditional refined sugar;
- sales revenues of DRD refined are higher than of raw sugar;

So, with the implementation of DRD we can have the following results:

a) For the sugar mill that had a traditional refinery, DRD will represent cost reduction of the product. In this case, the required investment to implement DRD corresponds to the sum of 3 values: disassemble of a portion of the sugar process in the mill, disassemble of the existing refinery, and of the resources applied in the implementation of the DRD process. With the cost reduction of the refined sugar, in Brazil payback of such total investment is about 3 milling seasons, for a volume of 10,000 bags of 50 kg of refined sugar /day, 180-days production;
b) A raw sugar producing mill, when implementing DRD process has the advantage of receiving the "premium", i.e., an additional amount paid for the refined sugar over the raw sugar, crystal or VHP. Figures 8 and 9 show the "premiums" that are effective in the sugar market in the periods indicated. Figure 9 shows the "premiums" of "Refined x Crystal" and "Crystal x VHP". The "premium" for "Refined x VHP" is the sum of the two former "premiums".
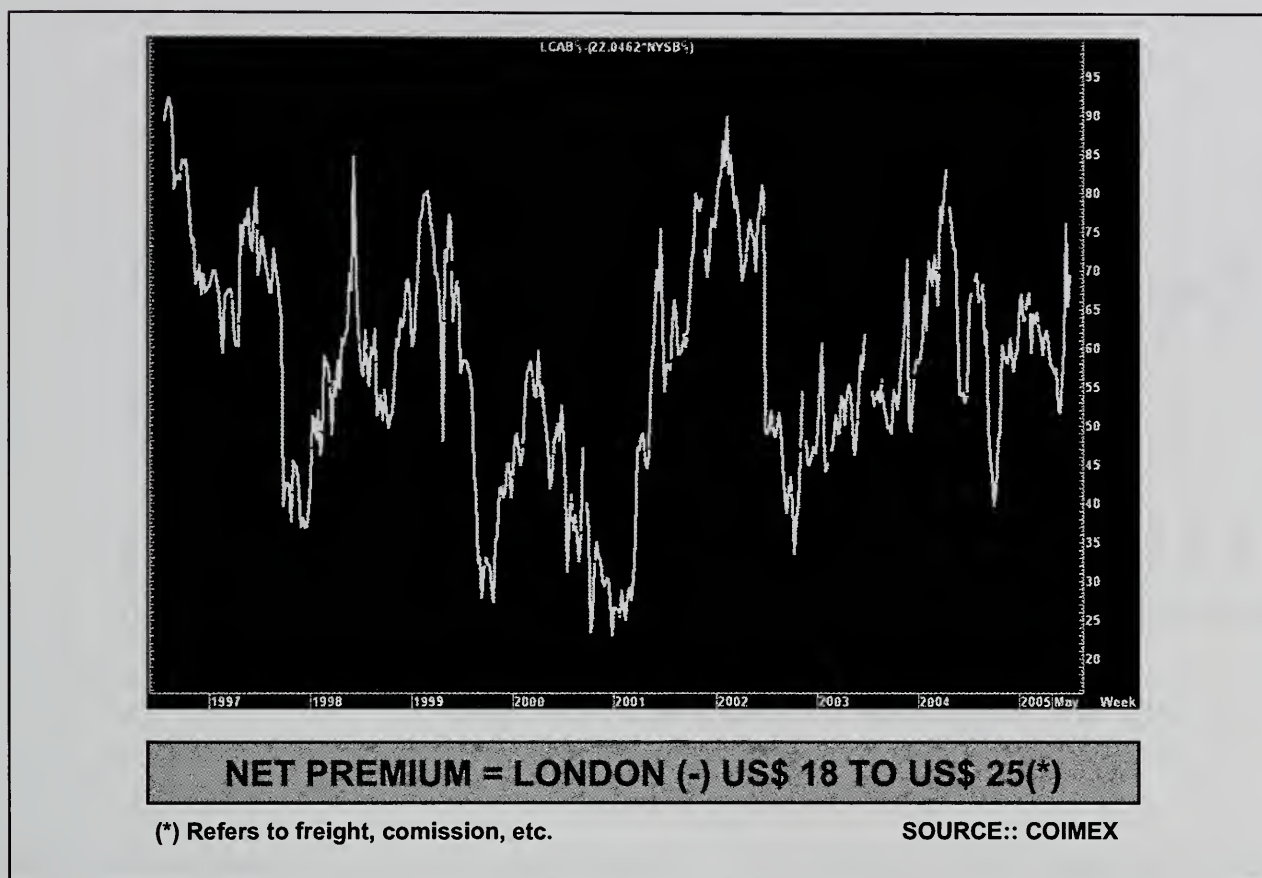
Figure 8.

In this example, the required investment to implement the DRD process corresponds to the cost of changes in the existing process of raw sugar, added by the values for implementing the required additional equipment. By using the average "premiums" of Figure 9, the above investment will have a payback period of about 2 milling seasons, in Brazil, in the same conditions of the previous item.

This same mill, when implementing the DRD process, by maintaining its capacity for the other types of sugar that it produces, may choose to sell the additional refined without reducing sales of raw sugar. In this case, payback will be even better.

Conclusions

The future international scenario shows an increasing demand for sugar, which has been higher than the population growth. Such increasing demand represents an opportunity for mills expansion. It should be noted that higher increase of production has already taken place in the most competitive countries, mainly due to their lower production cost.

As part of this scenario, a tendency that has been shown has become reality: the reduction of sugar production in countries where the subsidy represents unfair competition of the international trade. Such diminished production is also an opportunity because it opens space for demand to be filled by the most competitive mills.
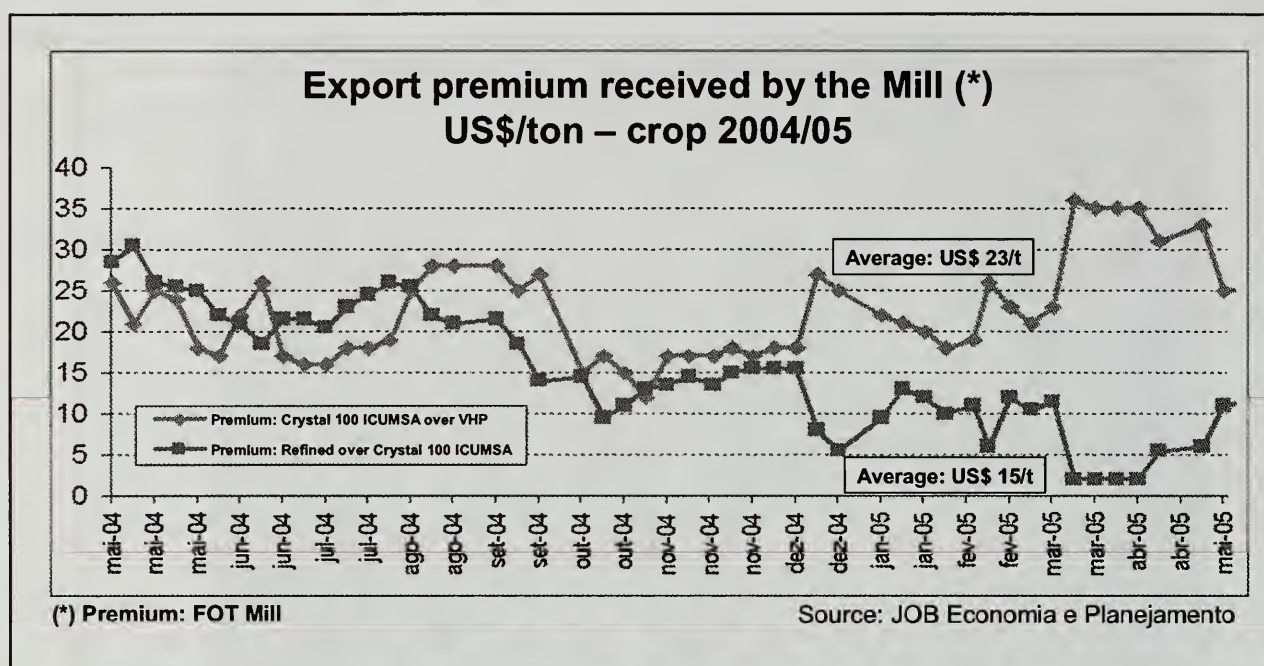
Figure 9. Premium: Crystal over VHP and White over Crystal

DRD – the refined sugar without the refinery – is aligned with these market prospects and will be benefited by enabling the production of refined sugar at cheaper cost by the mill itself.

So, we are convinced of the successful commercial introduction of this new technology and pleased for the opportunity that allows Dedini to contribute to the development of the sugar industry.

Acknowledgements

Our thanks to the mills that allowed and participated of the tests necessary to develop DRD technology, to Mr. Júlio Maria Borges, head of JOB Economia e Planejamento, expert on sugar international trading and economics, for his explanations and for figure 9 contribution, similar thanks to Mr. João Carlos Hopp Jr, director of Coimex, and thanks, too, to Rohm and Haas for the participation in the development of the second module of DRD process. Rohm & Haas, a worldly renowned company, has proved to have the ideal qualities for a partnership: knowledge, good will, innovation, creativity and persistence. Their work represents an important contribution to the success of DRD development.

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